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UNIVERSITY OF ALBERTA

THE PHARMACOLOGICAL EVALUATION OF NOVEL PYRIDYL DERIVATIVES OF CIMETIDINE

by

© S.B. TANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

Spring, 1990

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for a acceptance, a thesis entitled THE PHARMACOLOGICAL EVALUATION OF NOVEL PYRIDYL DERIVATIVES OF CIMETIDINE submitted by SHOU-BAI TANG in partial fulfilment of the requirement for the degree of MASTER OF SCIENCE in PHARMACOLOGY.

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MW Wolowyk
Supervisor

.....
CT Kappagoda

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DA Cook

Date: December, 20, 1989

To My Wife and Parents

ABSTRACT

Histamine H_2-receptor antagonists, cimetidine and related compounds, have been successfully used to treat peptic ulcer disease and have undoubtedly improved the quality of life for millions of patients worldwide. This has provided the stimulus to develop improved H_2-receptor antagonists which are more effective, than cimetidine, in suppressing nocturnal acid secretion and exhibiting a longer duration of action.

In attempting to design novel histamine H_2-receptor antagonists, a new class of pyridyl derivatives of cimetidine has been synthesized and evaluated in vitro on the histamine induced guinea pig (GP) right atrial chronotropic response. All of these compounds were found to antagonize the histamine H_2-receptor mediated response and a few of them exhibited potencies approaching that of cimetidine. The structure-activity relationship of these compounds were analysed.

However, the nature of inhibition by the novel pyridyl derivatives was noncompetitive, suggesting a different mechanism of action. To explore the possible mechanisms of action of these compounds, one of the most potent compounds, 2-{[2-[(4-pyridyl)methylthio]ethylamino]}-3-nitro-6-methoxypyridine (VJ-37; ID_{50} = 7.05 x 10^{-5} M), was further evaluated. The effects of VJ-37 on the positive chronotropic responses of isoproterenol, 8-bromo-cyclic AMP, milrinone and caffeine in GP

right atria and on the tonic responses of carbachol and $CaCl_2$ in guinea pig ileal longitudinal smooth muscle were also noncompetitive in nature. These results suggest that VJ-37 does not act via a direct action involving the histamine H_2-receptor, β-adrenergic receptor, muscarinic receptor, intracellular cyclic AMP, as well as sarcoplasmic reticulum mediated mechanisms. An action involving voltage sensitive or receptor operated calcium channels remains to be further investigated.

Nevertheless, VJ-37 did antagonize the gastric acid secretion in the pylorus-ligated rat *in vivo*, with the same potency as that of cimetidine. The possibility exists that VJ-37 may act beyond the points we investigated along the signal transduction pathways, e.g. calmodulin, contractile proteins, Ca^{2+} resequestration, Ca^{2+} extrusion, or may act at other unknown receptors, receptor subtypes, enzymes, or even proton pumps which can stimulate gastric acid secretion. The results indicate that VJ-37 can inhibit histamine H_2-receptor mediated responses *in vitro* and *in vivo* by a mechanism which differs from that of cimetidine.

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ABBREVIATIONS AND DEFINITIONS

AP	[^{14}C]aminopyrine
8-bromo-cyclic AMP	8-bromoinosine 3′,5′-cyclic monophosphate
Ca^{2+}	calcium ion
carbachol	carbamylcholine chloride
$CHNO_2$	2-nitro-1,1-ethenediamine
cyclic AMP	cyclic adenosine monophosphate
dbcAMP	dibutyryl cyclic adenosine monophosphate
DMSO	dimethylsulphoxide
DPH	diphenhydramine
DRC	dose-response curve
ED_{50}	the dose to obtain 50% of the maximal tissue response
EGF	epidermal growth factor
SK&F	Smith Kline & French Laboratories
G_i	inhibitory guanosine triphosphate-binding protein of adenylate cyclase
GPILSM	guinea pig ileal longitudinal smooth muscle
G_s	stimulatory guanosine triphosphate-binding protein of adenylate cyclase
HEPES	N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid
Het	heterocyclic
H^+/K^+ ATPase	hydrpgen-potassium adenosine triphosphatase
HPSS	HEPES buffered physiological saline solution

HR	heart rate
ID_{50}	the dose to obtain 50% inhibition of the maximal tissue response
K^+	potassium ion
K_a	association constant of an agonist
K_b	dissociation constant of an antagonist
NCN	cyanoguanidine
O_2	oxygen
pA_2	the negative logarithm of the molar concentration of a competitive antagonist which will reduce the response of a tissue to a double dose of agonist, to that of a single dose of agonist
PDE	phosphodiesterase
polygraph	high gain pre-amplifier recorder
Q tip	cotton-tipped applicator
SA cell	sinoatrial cell
SARs	structure-activity relationships
Schild plot	the method of plotting the dose shift ratio minus one (DR-1) against the negative log of the antagonist concentration (-log B, pA_x)
S.E.M.	standard error of the mean
SR	sarcoplasmic reticulum
VJ-37	2-{[2-[(4-pyridyl)methylthio]ethylamino]}-3-nitro-6-methoxypyridine

CHAPTER I

INTRODUCTION

More than 80 years have passed since the first synthesis of histamine (β-imidazolyethylamine) by Windaus and Vogt in 1907, but it was not until 1910 that Dale and Laidlaw demonstrated its dramatic and widespread physiological effects (Jones, 1966). Since then, histamine has been shown to have profound actions on most tissues and organs, both physiologically and pathologically. These included cardiac, skin, stomach, and brain tissues (Burland and Mills, 1982). It was also found that this amine was a natural constituent of animal body tissues in 1927 by Best and colleagues (Jones, 1966). After that, much information has been obtained about its broad distribution, as well as its synthesis, storage, metabolism and release.

Histamine had been relatively neglected by scientists in the last several decades in comparison with the effort expended on other endogenous substances such as the catecholamines, indolylalkylamines, and peptides. The reason for the neglect was partly due to the paucity of suitable histochemical research methods and the shortage of powerful agents to specifically block the synthesis, storage, metabolism, release and the target receptors of histamine.

In recent years, however, histamine research has experienced a rebirth because of some new developments in this area. The hypothesis of the existence of the histamine H_2-receptor and the discovery of H_2-receptor antagonists in the 1970's provided the tools to block almost all the effects of histamine (Black et al., 1972). With the introduction of a monoclonal antibody against L-histidine decarboxylase, histamine-synthesising and storing cells can be visualized by immunohistochemical methods (Watanabe et al., 1985; Pollard et al., 1985). Using radioligands such as [^{3}H]histidine and [^{3}H]histamine, the pharmacological properties of histamine receptors were revealed (Rising and Norris, 1985; Wells et al., 1985). The identification of the histamine H_3-receptor and the availability of its agonist, (R)-α-methylhistamine, together with its competitive antagonist, thioperamide, led to postulation of the negative feedback control of both histamine synthesis and release (Arrang et al., 1987). All these developments have resulted in a better understanding of histamine biosynthesis, metabolism, release, and target receptors, and have definitely enriched histamine research.

1.1 Histamine receptors and their classification

It has been well established that many drugs and hormones produce their actions by combining with a specific structure located on or within the surface of macromolecules or cells. This structure with which an agonist combined to produce the characteristic response of the cell to that agonist was named the

"receptor" (Furchgott, 1964). Receptors which are specific for certain agonists, including histamine, have been demonstrated through a variety of pharmacological methods, such as quantitative measurement of agonist potency ratios, selective agonism, selective receptor desensitization, differentiation of affinity and efficacy, and classification by antagonist interaction (Kenakin, 1987). Receptors can also be identified by some nonpharmacological methods, e.g., ligand binding, immunological cloning and gene recombination. Among all the above methods, analyses with competitive antagonists have provided the framework in which most of the existing receptors have been discovered. This was because the measurement of the equilibrium dissociation constants for competitive antagonist-receptor complexes (K_B) theoretically was more simple than for agonist receptor complexes (K_A) and, as well, the Schild plot [the method of plotting the dose shift ratio minus one (DR-1) against the negative log of the antagonist concentration (-log B, pA_x)] was a powerful tool with which to measure antagonist K_B values (Kenakin, 1987).

1.1.1 Histamine H_1-receptor

The first group of antihistamine substances were discovered by Bovet and Staub in 1937 (Durant et al., 1985) when it was reported that piperoxan and related aryl ethers such as 929F protected guinea pigs against lethal doses of histamine. Further work at the Pasteur Institute prompted intensive research activity in Europe and the United States to discover clinically effective antihistamines.

Histamine antagonists have been available since late the 1940's and these substances led to the identification of histamine receptors in many tissues. These receptors came to be known as histamine H_1-receptors. Some notable H_1-receptor antagonists discovered at that time include mepyramine, diphenhydramine, chlorpheniramine, triprolidine and promethazine (Durant et al., 1985).

1.1.2 Histamine H_2-receptor

It soon became apparent that the available antihistamines were not effective in blocking the histamine responses in every tissue. Responses to histamine in tissues such as guinea pig ileum and bronchial smooth muscle could be antagonized competitively by agents such as diphenhydramine and chlorpheniramine (Arunlakshana and Schild, 1959), but these antagonists were ineffective against the histamine responses in rat uterus, mammalian heart, and gastric acid secretion. It was also shown that histamine did not appear to be acting at a receptor for another agonist in these tissues (Ash and Schild, 1966; Trendelenburg, 1960; Ashford et al., 1949). After examining the blocking activity of the available histamine antagonists and the agonist activity of a series of histamine derivatives, Ash and Schild (1966) proposed that there were at least two types of histamine receptors and they defined the receptor which was competitively blocked by the classical antihistamines, such as those in guinea pig ileum and bronchial smooth muscle, as the "H_1" receptor. After that, Black and his co-workers (1972) succeeded in synthesizing a novel histamine antagonist, burimamide, which

competitively antagonized the histamine responses in rat uterus and guinea pig atrium, as well as the histamine-induced gastric acid secretion in rats and dogs. They called the histamine receptor in these tissues the "H_2" receptor. Later in 1970's, the relatively potent H_2-receptor antagonists, cimetidine and ranitidine, were developed and successfully used clinically on millions of patients worldwide in the treatment of peptic ulcer disease. Because of this remarkable contribution of drug development to modern medicine, Dr. Sir James Black was awarded the Nobel Prize for Physiology or Medicine in 1988 (Gerskowitch, et al., 1988; Black, 1989).

1.1.3 Histamine H_3-receptor

As histamine research continued, a third histamine receptor was identified. After studying histamine synthesis and release in rat and human brain tissue, it was found that histamine inhibited its own synthesis and release by a negative feedback process operating at the level of histaminergic nerve-endings (Arrang et al., 1983; Arrang et al., 1985; Arrang et al., 1987). Using R-α-methylhistamine as a selective agonist and thioperamide as a competitive antagonist respectively, Arrang and co-workers postulated (1987) that modulation of histamine release from cerebral neurons occurred via a negative feedback mechanism similar to that occurred in other aminergic systems and this was mediated by presynaptic histamine H_3-receptor. The H_3-receptor has been recognized as a multifunctional receptor with a widespread distribution (van der Werf and Timmerman, 1989). Activation of

H_3-receptors outside the central nervous system was shown to control mast cell histamine formation and release from the rat lung preparation (Arrang et al., 1987); to mediate serotonin uptake in human platelets (Gespach et al., 1986); and even to decrease postganglionic cholinergic-initiated contraction in guinea pig airways and ileum (Barnes and Ichinose, 1989; Ichinose and Barnes, 1989; Ichinose et al., 1989; Trzeciakowski, 1987; Tamura et al., 1988). In human bronchi in vitro, it was also demonstrated that α-methylhistamine similarly inhibited postganglionic cholinergic-induced contraction (Ichinose and Barnes, 1989). These findings implied that the H_3-receptor plays a modulatory role in airways and digestive tract and acts as a safety device to prevent malfunctions of airways and digestive tract, triggered by inappropriate release of excitatory neurotransmitters. Nevertheless, it is still not known whether the H_3-receptor effects observed in pharmacological models in vitro indicate that histamine mediates a tonic influence in normal or pathophysiological conditions in vivo. It is possible that a specific H_3-receptor agonist might offer an alternative mode for reducing hyperactivity of airways or intestines in allergic conditions. Even in the central nervous system, the function of cerebral histaminergic neurotransmission and its interaction with other neurotransmitter systems is still unknown (Van der Werf and Timmerman, 1989).

1.2 Histamine H_2-receptor antagonists and gastric acid secretion

Although the gastric secretory stimulant effect of histamine was described early in the present century, its possible role in the normal physiological control of gastric acid secretion was the subject of considerable controversy for quite a long time (Parsons, 1982). One difficulty that existed at that time was the lack of suitable pharmacological tools to dissect the problem. With the discovery of the histamine H_2-receptor antagonists, the antagonism of histamine H_2-receptors has assumed a key role in the pharmacological control of gastric acid secretion and peptic ulcer disease (Forte and Wolosin, 1987; Bertaccini and Coruzzi, 1988; Wolfe and Soll, 1988).

Elucidating the cellular mechanisms involved in gastric acid generation and secretion remains a difficult task because of the complexity of the regulatory processes involved. At the present time, it is considered that there are three distinctly different pathways involved in the delivery of chemical messengers to stimulate gastric acid secretion (Wolfe and Soll, 1988): the neurocrine pathway responsible for delivering transmitters, such as acetylcholine, which are released from postganglionic nerves in the stomach wall; the endocrine pathway answerable for releasing hormones, such as gastrin; and the paracrine pathway accountable for delivering tissue factors, such as histamine to the secretory cells. In addition, there are several hormones that might act

directly, via endocrine or paracrine pathways, to inhibit acid secretion from the parietal cell, such as epidermal growth factor (EGF), somatostatin and the prostaglandins (Berglindh, 1984).

Several reviews summarized our present understanding of how the gastric parietal cell functions (Berglindh and Sachs, 1985; Weir, 1988; Wolfe and Soll, 1988). According those reviews, the apical mucosal surface of the parietal cell was shown to contain a proton pump, the hydrogen-potassium adenosine triphosphatase (H^+/K^+ ATPase) enzyme, which, along with a carbonic anhydrase enzyme and a potassium chloride cotransporter, was responsible for potassium ion reabsorption from the lumen of the canaliculi into the cytosol in exchange for hydrogen. The energy for this process was provided by the hydrolysis of ATP (Lee, et al., 1974). H^+/K^+ ATPase could be inhibited by blocking the potassium chloride symport, by substituted benzimidazole such as omeprazole, and to a lesser extent by tricyclic antidepressant agents (Weir, 1988). The enzyme was stimulated by protein kinases, which were in turn activated by cyclic adenosine monophosphate (cyclic AMP); the cyclic AMP was generated from ATP by a catalytic subunit of adenylate cyclase; this catalytic subunit was linked to the histamine H_2-receptor by G_s, a stimulatory guanosine triphosphate-binding protein (Wolfe and Soll, 1988). Stimulation of the cyclase could be controlled by prostacyclin and prostaglandin E_2 which blocked the production of cyclic AMP (Soll, 1980). It was believed that prostaglandins acted through G_i, an inhibitory guanosine triphosphate-binding protein, to turn off the pump because the treatment of parietal cells with pertussis toxin markedly attenuated the prostaglandin inhibition of

histamine-stimulated function, without impairing the histamine-stimulated responses (Chen et al., 1988). By contrast, acetylcholine and gastrin were shown to stimulate H^+/K^+ ATPase through calcium dependent mechanisms because the stimulation was blocked by the removal of extracellular calcium or by the addition of lanthanum, a trivalent cation that blocks calcium fluxes across the plasma membrane (Soll, 1981). In this review, I will focus the role of the histamine H_2-receptor on the secretion of gastric acid.

1.2.1 Effect of histamine on gastric acid secretion

The role of histamine in the regulation of acid secretion has been controversial for decades (Waldum and Sandvik, 1989). However, the outstanding work of Black and co-workers (Black et al., 1972) provided evidence for the existence of histamine H_2-receptors in parietal cells and offered the possibility of blocking these receptors by the use of selective antagonists with consequent inhibition of gastric acid secretion. Resolution of the effect of histamine on gastric acid secretion required three major steps: localization of the receptors mediating the action of histamine on gastric acid secretion, identification of cells storing histamine in the fundic mucosa, and elucidation of the factors regulating histamine formation and release.

After years of studies, several lines of evidence indicate that parietal cells possess specific receptors for histamine, which are responsible for gastric acid secretion. The evidence is as

follows: 1) Histamine caused a time-dependent increase in [^{14}C]aminopyrine (AP) accumulation by rabbit, canine and human parietal cells, which is a measure of quantification of sequestered acid (Soll and Berglindh, 1987). 2) In studies with isolated dog parietal cells and gastric glands, histamine stimulated oxygen consumption, which directly correlated with the amount of HCl secreted and glucose oxidation, which were shown to be a determinant for the metabolic activity in parietal cells (Soll and Berglindh, 1987). 3) Histamine stimulated the measured acid secretion from rabbit gastric glands (Berglindh et al., 1976) and from parietal cells of the rat (Dial et al., 1981), guinea pig (Batzri and Dyer, 1981), frog (Michelangeli, 1976), and human (Soll and Berglindh, 1987). There were considerable species differences in the magnitude of the response to histamine. For example, histamine generally enhanced AP accumulation much more potently into rabbit than into canine parietal cells (Soll and Berglindh, 1987). 4) Although histamine alone stimulated parietal cell acid secretion, the response was enhanced by interaction with phosphodiesterase (PDE) inhibitors, such as isobutylmethylxanthine (Soll and Berglindh, 1987). 5) In examining a group of histamine analogues, a tight correlation was found between the ED_{50} (the dose which produces 50% of the maximal tissue response) for stimulation of AP accumulation and for stimulation of oxygen consumption (Chew et al., 1980), thus indicating an interaction at a common histamine receptor.

Despite the major role of histamine in the regulation of gastric acid secretion, the literature is incomplete on the identity of the fundic cells that store histamine and the regulation of histamine release. Defining the precise role of histamine has also been complicated by the fact that in the fundic mucosa, histamine is stored not only in mast cells, but also in endocrine-like cells. Furthermore, the proportion of these two kinds of histamine containing cells in the fundic mucosa varies widely among species (Soll and Berglindh, 1987). In certain species such as rat, rabbit and frog, a large proportion of fundic mucosal histamine was stored in endocrine-like cells that possessed acetylcholine and gastrin receptors too. Gastrin stimulated the formation of histamine by inducing the activity of histidine decarboxylase, and both acetylcholine and gastrin stimulated histamine release from these cells (Soll, 1985; Wolfe and Soll, 1988). In contrast to these findings, in humans and dogs, fundic mucosal histamine stores appeared to be fully accounted for by mast cells which also possessed receptors for prostaglandin E_2 and β_2-adrenergic agents, both of which inhibit the release of histamine; there was no data to indicate histamine storage in endocrine-like cells, and neither acetylcholine nor gastrin affected histamine release in this system (Soll and Berglindh, 1987; Wolfe and Soll, 1988). There is little disagreement that histamine is a regulator of parietal cell function. However, the nature of the regulation of histamine release from fundic mucosal stores remains poorly understood.

The complexity of the regulation of acid secretion reflects not only the fact that three pathways are involved, the histamine pathway as well as cholinergic and gastrin pathways, but also an interdependence between these pathways in all phases of acid secretion. This interdependence was clearly evident when specific histamine H_2-receptor antagonists and muscarinic receptor antagonists were shown to inhibit both the basal acid secretion and the stimulus-triggered acid secretion (Soll, 1985). Potentiation of interactions between secretagogues at the parietal cell were also reported. Data obtained from studies of both oxygen consumption (Soll, 1978) and AP accumulation (Soll, 1982) indicated that potentiating interactions occurred between histamine and carbachol, between histamine and gastrin, or when histamine, carbachol and gastrin were acting simultaneously (Soll, 1978). In dogs, the histamine H_2-receptor antagonist metiamide inhibited the response, not only to histamine but also to gastrin and cholinomimetics (Grossman and Konturek, 1974). Similarly, the anticholinergic agent atropine inhibited the response not only to cholinomimetics, but also to gastrin and histamine (Hirschowitz and Sachs, 1969). Also vagotomy decreased the response to both histamine and gastrin (Emas and Grossman, 1967). These findings led to the suggestion that during the basal state, the parietal cells are exposed to a constant background of acetylcholine and histamine, which sensitizes them to additional amounts of these agents, or of gastrin. Thus, the inhibition to the response of exogenous gastrin or histamine by atropine was interpreted to be a withdrawal of the sensitizing action of the basal level of acetylcholine. Similarly, the

inhibition to the response of exogenous gastrin and cholinomimetics was viewed as a removal of the potentiating action of the basal level of histamine.

Therefore the role of histamine in the regulation of gastric acid secretion still needs to be clarified. The old hypothesis by Grossman and Konturek (1974) that histamine might act as a final common mediator was strongly opposed by the following two types of recent experimental observations. The first piece of evidence derives from the studies of the ontogeny of gastric acid secretion, which indicated that in the rat, the receptors for gastrin and acetylcholine developed earlier than that for histamine (Ackerman, 1982). The second piece of evidence was the identification of the gastrin receptor on isolated canine parietal cells in vitro (Soll et al., 1984). Based on the data from the canine gastric cells, Soll proposed another model to describe the role of major pathways and receptors in gastric acid secretion in the fundic mucosa (Soll and Berglindh, 1987). According to this model, histamine, gastrin and acetylcholine act in parallel on specific receptors on the parietal cells, resulting in amplification of their actions through potentiating interactions. This hypothesis, recently called the "permission hypothesis" by Black and Shankley (1987), was challenged by the same author with the "transmission hypothesis". According to this hypothesis, besides the effects at their specific receptors on the parietal cells the main action of gastrin and acetylcholine was proposed to involve a release of histamine from endogenous stores. At present, both hypotheses are tenable, their acceptance being based on the different experimental models used. More work needs to

be done to elucidate the physiological role of histamine in the regulation of gastric acid secretion.

1.2.2 Mechanism of action of histamine to stimulate gastric acid secretion

The mechanism of action of histamine to stimulate gastric acid secretion is well established. The marvellous work by Black and colleagues developed a new era for histamine research when they discovered the first H_2-receptor antagonist, burimamide (Black et al., 1972). Since then, a lot of work has revealed the fact that the action of histamine on parietal cells is mediated by a pharmacologically typical H_2-receptor. Soll and Berglindh reviewed the following most important findings recently (1987). Histamine H_2-receptor antagonists competitively inhibited the histamine-stimulated increase of oxygen consumption and AP accumulation on rabbit gastric glands and on isolated canine, guinea pig and rat parietal cells (Soll and Berglindh, 1987). The pA_2 values (the negative logarithm of the concentration of a competitive antagonist which will reduce the response of a tissue to a double dose of of agonist, to that of a single dose of agonist) calculated from such data were 6.0 to 6.8, values similar to those obtained from guinea pig atrium and uterus (Soll and Berglindh, 1987). Anticholinergic agents, in concentrations that markedly inhibited the response to cholinomimetics, failed to shift the dose-response curve to histamine. Histamine H_1-receptor antagonists inhibited histamine-stimulated cyclic AMP production and AP accumulation (Soll

and Berglindh, 1987). However, these effects occurred at concentrations 10,000-fold greater than those necessary to inhibit typical H_1-receptors; at the same time, these effects were not specific for histamine, since the responses to carbachol and dibutyryl cyclic AMP (dbcAMP) were also blocked (Chew et al., 1980).

After histamine combines with H_2-receptor on the parietal cells, it is believed that it triggers cyclic-AMP-dependent activation mechanisms. Studies with isolated parietal cells and glands consistently indicated that histamine stimulated cyclic AMP production in parietal cells, in Necturus, rat, rabbit, guinea pig, dog and man (Code, 1982). It was reported that all analogues of histamine which stimulated gastric acid secretion simultaneously increased cyclic AMP levels and the increase could be blocked by H_2-receptor antagonists; but analogues which did not stimulate gastric acid secretion did not increase cyclic AMP levels (Scholes et al., 1976). The other supporting evidence included the findings that parietal cell function was activated by cyclic AMP analogues, phosphodiesterase (PDE) inhibitors, and forskolin, a diterpene that directly activates adenylate cyclase (Soll and Berglindh, 1987). Histamine H_1-receptor antagonists were found to inhibit histamine-stimulated cyclic AMP production; but in the case of AP accumulation, these effects were only found at concentrations 10,000-fold greater than those necessary to inhibit typical H_1-receptors (Chew et al., 1980).

In many cell types, an increase in cytosolic calcium appears to be the common mechanism of action of chemical transmitters to mediate cell activation. Such increase in cytosol calcium can be

achieved either by enhanced influx of extracellular calcium or by mobilization of intracellular calcium. The previous studies did not support the involvement of extracellular calcium in the stimulation of parietal cells by histamine, because the stimulation was only modestly impaired by removal of extracellular calcium, was not blocked by the inorganic calcium channel blocker, lanthanum, and was not associated with enhanced influx of $^{45}Ca^{2+}$ (Soll, 1985).

It is generally thought that cyclic AMP modulates cell function via cyclic AMP dependent protein kinase, the catalytic moiety of which in turn phosphorylates a target protein. Attempts to define this particular pathway in the parietal cell have met with limited success. Activation of the type I isoenzyme of protein kinase by histamine stimulation was demonstrated on partially purified rabbit parietal cells (Chew, 1985). It was also shown that histamine increased phosphorylation of two parietal cell proteins which were associated with cyclic AMP (Chew and Brown, 1987). The obvious, but difficult, next step would be to characterize the protein kinases whose phosphorylation is triggered by the action of histamine.

1.2.3 Inhibition of gastric acid secretion by histamine H_2-receptor antagonists

It was reported that histamine H_2-receptor antagonists selectively and competitively inhibited the stimulation by histamine at the H_2-receptor site of parietal cells (Burland and Mills, 1982). In addition, because of the interdependency among the pathways involved in hydrogen ion production, It was shown that

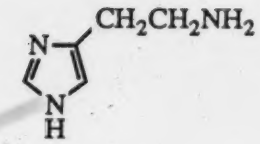
H_2-receptor antagonists could effectively reduce the acid secretion stimulated by gastrin, cholinergic agents, and food (Wolfe and Soll, 1988). It was also shown that histamine H_2-receptor antagonists reduced not only the volume of gastric juice but also the acid concentration and output, while having little effect on the secretion of pepsin (Robert, 1987).

In vitro studies with isolated oxyntic cells demonstrated that histamine was the most potent stimulus among three gastric acid secretagogues, histamine, gastrin and acetylcholine; inactivation of histamine markedly reduced the secretory effects of both acetylcholine and gastrin (Soll, 1978). Depending on the duration of action, different H_2-receptor antagonists produced gastric luminal anacidity for variable periods throughout twenty-four hours. Because there are three receptor types on parietal cells, it is not surprising that strong stimulations via the acetylcholine or gastrin pathway could break through H_2-receptor blockade (Berglindh and Sachs, 1985). Thus, it is difficult to achieve a twenty-four hour complete suppression of acid secretion by H_2-receptor antagonists.

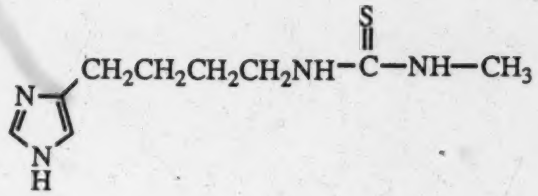
1.2.4 A survey of histamine H_2-receptor antagonists

The first compound to be described as histamine H_2-receptor antagonist was burimamide (Black et al., 1972). To synthesize burimamide, histamine was used as the chemical starting point and N^{α}-guanylhistamine was used as the "lead" compound (Ganellin, 1982; Black, 1989). Consequently, the first group of histamine

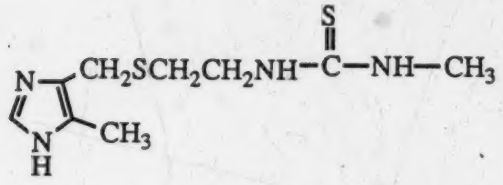
H_2-receptor antagonists developed were imidazole derivatives. Chemical modification of burimamide led to the synthesis of metiamide (Black et al., 1973) and cimetidine (Brimblecombe et al., 1975). In 1976, cimetidine was marketed for the treatment of conditions associated with gastric hyperacidity. Three years later, another two derivatives of cimetidine, tiotidine and ranitidine, were found to be potent histamine H_2-receptor antagonists. Possessing the same side chain as cimetidine, tiotidine had a guanidinothiazole substituted ring (Yellin et al., 1979) and ranitidine had a dimethylaminomethylfuran substituted ring (Bradshaw et al., 1979). Ranitidine became the second compound to be marketed. In contrast to previous views (Black, 1989), It was demonstrated that a basic imidazole ring was not essential for histamine H_2-receptor affinity and could be replaced using some aromatic heterocyclic rings. Later numerous chemically different histamine H_2-receptor antagonists were synthesized, bearing little or no structural resemblance to the original lead compound, N^{α}-guanylhistamine. Among these compounds, four were clinically tested and became the first generation of histamine H_2-receptor antagonists. They included burimamide, metiamide, cimetidine and ranitidine (figure 1.1). Burimamide had no future as an antisecretory drug because it was poorly absorbed by the intestine. After a year of clinical trials, the use of metiamide was discontinued, because a few patients developed agranulocytosis. Cimetidine and ranitidine, however, are still used worldwide (Robert, 1987).



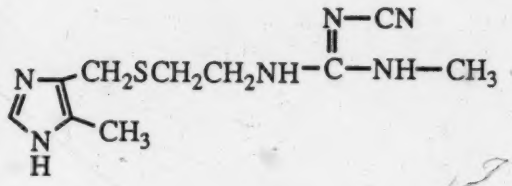
Histamine



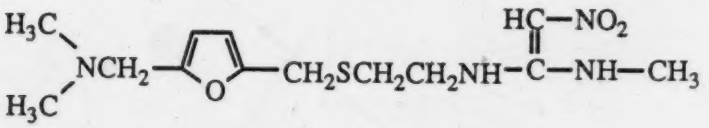
Burimamide



Metiamide



Cimetidine



Ranitidine

Figure 1.1 Structure of histamine and several first generation histamine H_2-receptor antagonists.

The discovery of the first generation of histamine H_2-receptor antagonists and their clinical success have provided both the rationale and stimulus to develop other histamine H_2-receptor antagonists with improved potency, a longer duration of action and a lower potential for side effects. Attempts to improve these pharmacological profiles have led the development of a number of second generation compounds. The second generation of histamine H_2-receptor antagonists that were tested clinically are listed in figure 1.2. All were claimed to be more potent than cimetidine as histamine H_2-receptor antagonist; other than that, SK&F 93479 (Smith Kline & French Laboratories) had a longer duration of action in vivo (Robert, 1987).

Classification of histamine H_2-receptor antagonists has been difficult because the compounds comprise such a diverse group both chemically and pharmacologically. Ganellin suggested (Ganellin, 1985) that they could be divided into two main structural classes: 1) compounds containing a flexible connecting chain, 2) compounds with conformationally restricted analogues or with diaryl structures. Based on the different aromatic rings, four main series can be described in the first group of compounds (figure 1.3). In relation to the different benzenoid structural substituents, three main series can be described in the second group of compounds (figure 1.4).

Figure 1.2 was removed due to the unavailability of copyright permission.

Figure 1.2 Structure of the second generation histamine H_2-receptor antagonists which were clinically tested (Robert, 1987).

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Figure 1.3 General formula of histamine H_2-receptor antagonists containing a flexible connecting chain (Ganellin, 1985). According to the aromatic ring, there are four main series.

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Figure 1.4 General formula of histamine H_2-receptor antagonists containing diaryl structures (Ganellin, 1985). According to replacement of benzenoid structure, there are three main series.

1.2.4.1. Histamine H_2-receptor antagonists containing a flexible connecting chain

The common structural features of this group of compounds are an aromatic ring system, a flexible connecting chain and a polar (uncharged at physiological pH) planar group (Figure 1.3). The compounds in this group can be subdivided into four main series according to the aromatic rings they possess: a) imidazole, b) aminomethylfuran, c) guanidinothiazole and d) aminomethylphenoxy series. Modifications to the flexible side chain, such as chain branching or replacing the sulfur atom by alternative heteroatom linkages, resulted in considerable reduction in activity (Brown and Young, 1985; Buschauer et al., 1989). Thus the different structural alterations were directed to the attachments at both ends of the flexible side chain, the aromatic ring at one end and the polar planar group at the other.

Within the imidazole series of histamine H_2-receptor antagonists, burimamide was the first compound developed, but because of its relatively low potency and poor oral bioavailability, it was not used clinically (Black, 1989). Inserting an electro-negative thioether linkage in place of the methylene group and introducing a 4-methyl group produced metiamide, which was much more potent and better absorbed than burimamide (Black, 1989). Bone marrow toxicity associated with the thiourea moiety was then eliminated by replacing the thiourea with a cyanoguanidine group. This resulted in the production of cimetidine (Brown and Young, 1985).

A number of attempts have been made to enhance the activity of cimetidine by structural modifications of the cyanoguanidine group. Numerous uncharged substituents with varying lipophilicity and dipole moment orientation, such as a 2-nitro-1,1-ethenediamine, 2-amino-3-nitropyrrol, 2-amino-4-pyrimidone (isocytosine), or a zwitterionic group were tried (Young et al., 1987; Brown et al., 1989; Buschauer et al., 1989). In general, replacement of the cyanoguanidine in cimetidine by groups other than neutral amidines led to the reduction of antagonistic activity (Brown and Young, 1985). The isocytosine system led to the enhancement of antagonistic activity if the pyrimidyl ring was substituted at the 5 position, such as oxmetidine. Oxmetidine was a noncompetitive antagonist about 16 times as potent as cimetidine in vitro and 2-3 times as potent in inhibiting gastric acid secretion but it was withdrawn owing to its toxic effects on the liver (Brown et al., 1988; Buschauer et al., 1989). Etintidine, an analog of cimetidine with a propargyl instead of a methyl substituent at the cyanoguanidine moiety, was found to be about two to three times more active than cimetidine in vitro (Cavanagh et al., 1983).

In the aminoalkylfuran series of histamine H_2-receptor antagonists, the optimal substitution pattern proved to be the 2,5-disubstituted heterocycle. It was found that the highest potency was obtained if the tertiary amine was a dimethylaminomethyl group (Buschauer et al., 1989). Ranitidine possessing the nitrodiaminoethene side chain of the cimetidine, while the basic imidazole ring was replaced by a furan ring, attached by a basic dimethylaminomethyl group (Bradshaw et al., 1979), was claimed to be

4 - 5 times as potent as cimetidine and to have a short duration of action. SARs among ranitidine analogues were reported to be different from those in the cimetidine series. The thiourea and cyanoguanidine analogues of ranitidine were considerably less active than ranitidine (Brown and Young, 1985). Replacement of the furan ring of ranitidine by thiazole produced the compound nizatidine, which was reported to be 5 - 18 times as potent as cimetidine *in vitro* (Lin et al., 1983).

The guanidinothiazole series comprised a group of compounds with extremely high H_2-receptor affinity. Representative in this group was tiotidine, which was about 5 times as potent as cimetidine and had a relatively slow onset of action and a longer duration of action (Yellin et al., 1979). The guanidinothiazole group has been incorporated into many histamine H_2-receptor antagonists, such as pyrimidones, thiazole oxide derivatives, 1,2,4,6-thiatriazine-1,1-dioxides, hydantoins, N-cyanamidines, N-carbamoylamidines, or sulfamoylamidines such as famotidine (Buschauer et al., 1989). Famotidine was reported to be about 50 - 70 times as potent as cimetidine *in vitro* and 40 -50 times as potent *in vivo*.

The discovery of the piperidinomethylphenoxy series as an alternative structure to imidazole rings with high affinity for histamine H_2-receptor has considerably enlarged the scope of modifications to the polar moiety. Numerous derivatives in this series produced noncompetitive (unsurmountable) antagonism in isolated guinea pig right atrium and were difficult to remove from the tissue by washing. The triazole derivatives lamtidine and loxitidine were very potent and extremely long-acting histamine

H_2-receptor antagonists (Buschauer et al., 1989). Unfortunately, it was shown that noncompetitive (unsurmountable) antagonists with an extended duration of action may induce mucosal changes. The trials with loxitidine were suspended because of its carcinogenic effect in the rat (Brittain et al., 1985).

In recent years, the search for further structural substituents of the polar group has expanded. Besides cyanoguanidines, nitroethenediamines, and some others, many novel "urea equivalents" have been incorporated into antagonist molecules. This included thieno-, benzoisothiazole-1,1-dioxide, isomeric thiadiazole diamines, oxadiazole diamines, diaminopyrimidines, diaminofurazan, and amines such as the nicotineamide-N-oxide derivative roxatidine (Sorba et al., 1985; Buschauer et al., 1989). The later compound was reported to be a competitive H_2-receptor antagonist about as potent as ranitidine, with a somewhat longer duration of action.

Histamine H_2-receptor antagonists with cyclic "urea equivalents" have also been well studied. Compounds where the aromatic ring (Het) was a pyridyl ring system and the polar group was 2-nitro-1,1-diaminoethene moiety, N-alkyl (cycloalkyl) thiourea and cyanoguanidine moiety, 3,4-amino-1,2,5-thiadiazole-1-oxide and 1,1-dioxide "urea equivalent" Y were reported to be histamine H_2-receptor antagonists (El-Badry et al., 1985; El-Badry et al., 1985b; Agrawal et al., 1987). The testing results suggested that the charge distribution in the Het pyridyl ring was important for interaction with the histamine H_2-receptor. It was, therefore, of interest to examine cyclic "urea equivalents" provided by the incorporation of cyanoguanidine, 2-nitro-1,1-ethenediamino,

2-amino-3-nitropyridyl, or 2-amino-3-nitro-1H-pyridyl-6-one as the Y component in this thesis.

1.2.4.2 Histamine H_2-receptor antagonists with diaryl structures

A further development in structural augmentation was the discovery that the flexible connecting chain could be replaced by a more rigid benzenoid structure to produce active diaryl compounds (Figure 1.4). The first type of derivatives were the meta-phenylene analogues of methylthiourea and diamino-nitroethene. There also appeared to be a considerable degree of stereochemical specificity since ortho- and para-phenylene isomers of the methylthiourea were not active (Ganellin, 1985).

When the imidazole ring was retained, but the flexible chain was replaced by phenylene (e.g. mifentidine), histamine H_2-receptor antagonist activity increased by about 30 times relative to that of cimetidine (Donetti et al 1984).

The third type of diaryl structure made by replacement of the pyridyltriazoles, resulted in a more potent compound than cimetidine in vitro (Buschauer et al., 1989).

1.2.5 Therapeutic applications of Histamine H_2-receptor antagonists

The H_2-receptor antagonists which have be ı most commonly available for safe clinical use are cimetidine and ranitidine. They

have been prescribed to over forty million and fifteen million people respectively; and the paucity of side effects has been dramatic (Weir, 1988). Their major clinical applications have been for acid-hypersecretion-related disorders, such as peptic ulcer, Zollinger-Ellison syndrome, and antral G-cell hyperplasia.

Ulcers are defects in the gastrointestinal mucosa that penetrate the muscularis mucosa (Richardson, 1988). In general, an ulcer can be controlled by reducing the amount of acid which reaches the ulcer. The aim of ulcer treatment has often been to decrease the gastric acid secretion. With the use of H_2-receptor antagonists, cimetidine and ranitidine, acid secretion could be effectively and safely suppressed and in the vast majority of patients, peptic ulcer could be healed medically (Wolfe and Soll, 1988). The availability of the histamine H_2-receptor antagonists has marked a new era in the treatment of acid-related peptic disorders.

Two other diseases characterized by duodenal ulceration and hypersecretion of gastric acid are the Zollinger-Ellison syndrome and antral G-cell hyperplasia. The Zollinger-Ellison syndrome was characterized by non-β-islet-cell tumors that secreted gastrin (Wolfe and Jensen, 1987). Antral G-cell hyperplasia was characterized by moderately elevated fasting serum gastrin concentrations with exaggerated postprandial increases in serum gastrin levels (Glowniak et al., 1982). There was an increase in the density of antral gastrin cells, but no sign of gastrin secreting tumors (Friesen and Tomita, 1981). Cimetidine has been shown to be effective in the treatment of those two kinds of patients, providing complete symptomatical relief (Burland and

Mills, 1982). The same author also indicated that cimetidine could be effective in other hypersecretory states, such as hypertrophic hypersecretory gastropathy and short bowel syndrome.

Other clinical uses of histamine H_2-receptor antagonists are reflex esophagitis, stress ulcer in the severely ill or burned patient, upper gastrointestinal bleeding (although this use is controversial), and preanaethetic use in emergency operations to lower the incidence of acid aspiration syndrome (Douglas, 1985).

1.3 Chronotropic effect of guinea pig right atrium - a standard test model for histamine H_2-receptor antagonist screening

Modification of the cardiac rhythm by histamine has been appreciated almost since the compound's isolation from ergot by Barger and Dale in 1910 (Wolff and Levi, 1986). The observation that histamine caused an increase in sinoatrial rate of the isolated cardiac preparation of the guinea pig and the fact that this response was selectively antagonized by histamine H_2-receptor antagonists has formed the basis of an important technique for the identification and quantitative evaluation of compounds which act at the histamine H_2-receptor. (Black et al., 1972; Levi et al., 1976). Studies on the cardiac effects of histamine have further contributed to the classification of histamine receptors and the development of novel histamine H_2-receptor antagonists.

1.3.1 The positive chronotropic effect of histamine on guinea pig right atrium

Histamine increased the sinus rate of the isolated heart of the guinea pig and this effect could not be antagonized by classical antihistamines in the concentrations that could sufficiently block H_1-receptors (Levi et at., 1982; McNeill, 1984; Wolff and Levi, 1986). Because classical antihistamines failed to selectively antagonize the cardiostimulatory actions of histamine, it was thought that histamine-induced cardiac stimulation might result from catecholamine release (von Euler, 1966). Various experiments, however, demonstrated that the chronotropic response to histamine was preserved despite the depletion of catecholamine, the destruction of adrenergic terminals or the blockade of β-receptors (Wolff and Levi, 1986). After the discovery of H_2-receptor antagonist by Black and co-workers, the increase in heart rate was shown to be competitively antagonized by H_2-receptor antagonists, such as metiamide or cimetidine; the pA_2 values calculated from the data were 5.1 to 5.3, and which were similar to those obtained from rat gastric acid secretory experiments (Black et al., 1972; Levi et al., 1982). Furthermore, the chronotropic effect of histamine was mimicked only by selective H_2-receptor agonists such as 4-methylhistamine or dimaprit, which were competitively antagonized by metiamide and cimetidine; and were not mimicked by the selective H_1-receptor agonists, 2-methylethylamine or 2-thiozolylethylamine (Humphary et al., 1982; Levi et al., 1975; Levi et al., 1982; Verma and McNeill, 1976).

Thus it seems certain that the positive chronotropic effect of histamine in the guinea pig is mediated exclusively by the histamine H_2-receptor.

1.3.2 The mechanism of histamine H_2-receptor mediated positive chronotropism on guinea pig right atrium

In recent years, a lot of work has been done to elucidate how the H_2-receptor mediates its positive chronotropic effect on guinea pig right atrium. It was assumed that after the activation of H_2-receptor, cyclic AMP dependent activation mechanisms were initiated. Verma and McNeill (1977) showed that histamine or the H_2-receptor agonist 4-methylhistamine increased cyclic AMP levels prior to chronotropism in guinea pig right atria; these effects were also shown to be antagonized by the histamine H_2-receptor antagonist burimamide. This work was confirmed by Trist and colleagues (1987). Theophylline and 3-isobutyl-methylxanthine, two PDE inhibitors, potentiated the guinea pig chronotropic response to histamine (Broadley and Wilson, 1980). It was further found that papaverine, another PDE inhibitor, potentiated the effect of histamine on the spontaneous rate of the guinea pig right atrium (Levi, 1982). Although more extensive work was still required, these early experiments supported the view that cyclic AMP was involved in the chronotropic effect of histamine on guinea pig right atrium.

The effects of histamine on transmembrane electrical activity of guinea pig sinoatrial node has been studied as well. Pacemaker activity in the sinoatrial node results from rhythmic, automatic oscillations of the membrane potential (Gilmour and Zipes, 1986). During diastole, the membrane potential slowly decreases, commonly designated as phase 4 depolarization, to a threshold level from which the propagated action potential is generated. Heart rate is dependent on the rate of the diastolic depolarization, an increase in the slope of phase 4 depolarization resulting in an increase in heart rate.

The mechanism by which histamine increases the sinus rate appears to be due to a marked enhancement of the slope of phase 4 depolarization. Levi and Pappano (1978) observed by microelectrode methods that the rate of spontaneous diastolic depolarization could be augmented by histamine without affecting the maximum diastolic potential, the threshold potential, amplitude, or duration of the action potential. The effect was enhanced by increasing concentrations of external calcium and was reversed by cimetidine. Histamine could restore the negative chronotropic effect of high concentration of extracellular potassium. This restoration was competitively inhibited by the H_2-receptor antagonist burimamide. The action of histamine on phase 4 depolarization was not affected by reserpine pretreatment or by propranolol pretreatment (Levi, 1982). On the other hand, a recent study from a Mexican research group, using microelectrode methods, showed that histamine increased not only the rate of diastolic depolarization, but also the maximum diastolic potential, the amplitude and maximum rate of

depolarization of the action potential of guinea pig pacemaker cells. These effects were also antagonized by the H_2-receptor antagonist cimetidine, but not by the H_1-receptor antagonist chlorphenamine (Sanchez-Chapula and Elizalde, 1987). Although there were disagreements on how many effects histamine had on pacemaker cells, everyone seemed to agree that histamine increased heart rate by enhancing the rate of diastolic depolarization which was mediated by the H_2-receptor.

1.3.3 Guinea pig right atrium, one of the best tissues for histamine H_2-receptor antagonist screening

The guinea pig has been a commonly used animal species for experimental purposes for decades. In histamine research, most publications reported use of the guinea pig, partly because this animal has a high sensitivity to anaphylaxis which is one of the most severe pathological responses of histamine (Levi et al., 1982). As a result, the effects of histamine on guinea pig heart have been well described and studied in great detail in comparison to that of other species.

Cardiac histamine concentration and effects of histamine on the heart were found to be species dependent. "The guinea pig seemed to have been designed by nature for the delight of those pharmacologists interested in histamine" (Cook, 1984) for the following reasons: 1) It was shown that there was an uneven distribution of histamine stores in different parts of the guinea pig heart; more histamine was found in the atria than in the

ventricles, and in the right atrium than in the left (Giotti et al., 1966; Assem et al., 1987), making the right atrium a sensitive tissue for study of histamine release. 2) The in vivo stimulatory effects of histamine on animal hearts were composed of both direct and indirect effects, the direct effect was mediated by cardiac histamine receptors and the indirect effect was due to the fall in blood pressure and the release of catecholamines from the adrenal medulla (Levi et al., 1982). It was revealed that in guinea pig, the direct cardiac effects predominated, with little interference from the indirect effects; while in some other species, the direct effect of histamine on the heart was greatly overshadowed by the indirect effects, e.g., baroreceptor reflex response predominated in the dog and catecholamine release prevailed in the cat (Levi, 1982). 3) Guinea pig cardiac responses to histamine were found to be quite similar to that of the primates and man (Levi et al., 1979). 4) Types of cardiac histamine receptors are different among different species. It was confirmed that only H_2-receptor existed in guinea pig right atrium; but for some other species such as rabbit, both H_1- and H_2-receptors coexisted (McNeill, 1984), making such species inappropriate for H_2-receptor antagonist screening.

Because of the characteristics of the guinea pig right atrium, especially the fact that the histamine-mediated response in the atrium is clearly a H_2-receptor-mediated response, this in vitro preparation has become accepted as the best way to test novel histamine H_2-receptor antagonists.

CHAPTER II

OBJECTIVE OF THESIS

The definition and characterization of the H_2-receptor using burimamide paved the way for the discovery of cimetidine and its subsequent use for treating peptic ulcer disease. These drugs and their successive generations, e.g., ranitidine, have undoubtedly improved the quality of life for millions of patients worldwide. This has provided the stimulus to develop improved H_2-receptor antagonists which are more effective in suppressing nocturnal acid secretion and exhibiting a longer duration of action, than ranitidine and cimetidine, and which have fewer side effects.

Discoveries of second-generation histamine H_2-receptor antagonists with different chemical structures have increased dramatically in the last decade. In the Smith Kline and French Laboratories, approximately 700 compounds were synthesized and assayed (Gerskowitch et al., 1988). None of these compounds was found to be clinically useful. In a recent review by Robert (1987), it was pointed out that some second generation H_2-receptor antagonists were not potent enough to replace ranitidine, such as etintidine and oxmetidine; and others were abandoned because of their dysplastic effect or gastric carcinogenesis in rats, such as SKF 93479 and tiotidine. Famotidine was the only compound found to be more potent than ranitidine, had longer duration of action and showed no signs of dysplastic or carcinogenic effects.

Attempts to develop compounds with greater potency and longer duration of action, yet devoid of fatal side effects from the research interest of two medicinal chemists, Drs. E.E. Knaus and V.K. Agrawal. Using the fundamental structural features of the first generation histamine H_2-receptor antagonists, cimetidine and ranitidine, they have synthesized 58 novel compounds (Agrawal et al., 1988; Agrawal et al., accepted). The pharmacological evaluation of 30 of these compounds is presented in this thesis. The primary goals of this study were twofold: 1) to accomplish a systematic pharmacological characterization of the SARs of novel derivatives of cimetidine and ranitidine, 2) to elucidate the possible mechanism of action of one of the most potent compounds in these derivatives, 2-{[2-[(4-pyridyl)methylthio]-ethylamino]}-3-nitro-6-methoxypyridine (VJ-37).

The specific objectives of this thesis were therefore to use isolated tissues and intact animals:

1. to quantitate the pharmacological profiles of new derivatives of cimetidine and ranitidine on guinea pig right atrium by using standard pharmacological screening tests *in vitro*.
2. to establish some SARs of the novel compounds on the guinea pig right atrium.
3. to clarify the possible mechanisms of action of VJ-37 by using different *in vitro* and *in vivo* experimental models.

CHAPTER III

MATERIALS AND METHODS

3.1 Standard drugs and novel compounds

Atropine (sulfate salt), 8-bromoinosine 3′:5′-cyclic monophosphate (8-bromo-cyclic AMP), caffeine (anhydrous), cimetidine, diphenhydramine (HCl), forskolin, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), histamine (dihydrochloride), and isoproterenol were all purchased from Sigma Chemical Co., U.S.A.

Calcium chloride (anhydrous), D-glucose, magnesium chloride, and potassium chloride were all obtained from Fisher Scientific Co. Ltd., Canada.

Dimethylsulphoxide (DMSO), polyethylene glycol 400 (PEG) and sodium chloride were provided by British Drug Houses Ltd., Canada. Collodion and sodium hydroxide were provided by J.T. Baker Chemical Co., U.S.A.

The following chemicals were also used: Bay K 8644 (Dr. Knaus' Medicinal Chemistry Lab, University of Alberta, Canada), carbamylcholine chloride (carbachol, Aldrich Chemical Company Inc., U.S.A.), ethyl ether (Caledon Laboratories Ltd., Canada), milrinone (Sterline-Winthrop Research Institute, U.S.A.), nadolol (Squibb, Canada).

All novel compounds tested were synthesized in Dr. E.E. Knaus' medicinal chemistry laboratories at the University of Alberta by Dr. V.K. Agrawal.

3.2 Physiological solutions

All solutions used were physiologically isotonic with animal tissues. For most in vitro experiments, HEPES buffered physiological saline solution (HPSS) of the following composition in mM/L was used: NaCl 135.0, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 2.1, HEPES 10.0, D-glucose 10.0, and adjusted to PH 7.4 by 2 M NaOH aerated with 100% oxygen (O_2). In solutions used for the study of calcium (Ca^{2+}) dose-response-curve (DRC) on guinea pig ileal longitudinal smooth muscle (GPILSM), Ca^{2+}-free HPSS was prepared by equimolar substitution of NaCl for $CaCl_2$ in the HPSS. In order to achieve Ca^{2+} DRCs on GPILSM in the same experiment, 93.9 mM Ca^{2+} solution was prepared by equimolar substitution of $CaCl_2$ for NaCl in the HPSS. For experiments involving potassium (K^+)-induced contraction on GPILSM, 142.9 mM K^+ solution was made by equimolar substitution of KCl for NaCl.

In guinea pig in vitro experiments, a large number of the drugs were dissolved in HPSS, although 100% DMSO were used for dissolving 10^{-1} M cimetidine, forskolin, milrinone, nadolol and all of the synthesized compounds because they could not be dissolved by HPSS or double distilled water; the solutions were then serially diluted with HPSS.

In the *in vivo* rat gastric acid secretory experiment, isotonic saline solution was made up by 0.9 g NaCl in 100 ml double distilled water for dissolving different drugs.

3.3 *In vitro* guinea pig right atrial experiment

Male Hartley strain guinea pigs (Charles River Canada Inc., St. Constant, Quebec) weighing between 350 - 700 g were sacrificed by stunning and exsanguination. Working as quickly as possible, the thorax was opened and the heart was exposed, removed, and plunged into HPSS, which had been aerated with 100% O_2, at 37°C. All other tissues were then cut away until only the right atrium was left. A thread was attached to each longitudinal end of the atrium and the tissue was suspended in a tissue bath of 10 ml capacity (figure 3.1). One end of the atrium was fixed by a thread to the bottom of the bath and the other was attached to a force-displacement transducer (Model FT03; Grass). The transducer was then coupled to a high gain pre-amplifier recorder (polygraph; Model 7D; Grass), by which isometric tension responses of the tissues were recorded. Before recording, the signals had been magnified several times by pre-amplifiers (Model 7P1G; Grass) and driver amplifiers (Model 7DAG; Grass) in the polygraph. The bath was filled with HPSS, maintained at 37°C by a water circulator (Model E52; Haake or Thermomix Model II; B. Braun) and was aerated with 100% O_2.

Figure 3.1 was removed due to the unavailability of copyright permission.

Figure 3.1 Isolated tissue set-up for guinea pig right atrium and GPILSM (Matowe, 1989).

Immediately after mounting the right atrium, the spontaneous heart contraction was recorded. The atrium was allowed to equilibrate for about one hour under a tension of 0.25 g with changes of HPSS every 15 minutes. During this time, the chart speed was set at 0.5 cm/min. Optimal tension-contraction relationships were determined for each tissue prior to beginning each experiment. The tension was then set to 75 - 85% of the maximal contraction, usually between 0.75 - 1.00 g. Subsequently, heart rate measurements were recorded at a chart speed of 30 cm/min.

3.3.1 Experimental protocols for evaluating novel compounds on guinea pig right atrium

The following experimental protocols were used for screening novel compounds and for exploring the mechanisms of action of VJ-37, one of most potent novel compounds, in guinea pig right atrium:

1. After the determination of the optimal resting tension, a cumulative DRC of the agonist was prepared. The drugs were added in a geometrical fashion, increasing concentration in steps of half-log units. The maximal response of the tissue to each dose of agonist was allowed to develop and plateau (about 2 min/dose) prior to administration of the next higher dose. Once the maximal response to agonist had been obtained, cumulative additions (about 3 min/ dose) of the test compound (antagonist) were used to obtain an inhibitory DRC. Administration of test compound was stopped once the the ID_{50} (the dose to produce 50% inhibition of the maximal tissue response) was attained.

2. The tissue was washed three times with HPSS and permitted to re-equilibrate for 15 minutes. A challenge maximal dose of the agonist histamine, or some other positive chronotropes, was then given to see whether the tissue recovered from the previous antagonist treatment. The tissue was then washed three times again.
3. A dose of antagonist, which was predetermined to be less than the ID_{50}, was added to the tissue. 30 minutes later, another cumulative DRC of agonist was obtained.
4. Step 2 was repeated.
5. Step 3 was repeated, with the dose of antagonist above the ID_{50}.

In most of the above experiments, the agonist was histamine; in experiments to elucidate the mechanism of action of VJ-37, the agonists were drugs which had a positive chronotropic effect, such as, isoproterenol or milrinone. In experiments which involved histamine, a concentration of 10^{-6} M nadolol was added 15 minutes before adding of histamine to the tissue bath.

3.4 <u>In vitro</u> guinea pig ileal longitudinal smooth muscle experiment

A guinea pig was sacrificed as mentioned above. The abdomen was opened longitudinally and the caecum was lifted and placed on the right hand side of the animal, exposing the ileo-caecal junction. The ileum was cut one to two centimetres above this junction and the mesenteric attachments were freed for about twenty centimetres.

Another cut was made at the end of the cleaned section to remove the the ileum and taking care not to stretch the tissue. The tissue was then immersed in HPSS that had been aerated with 100% O_2.

The method used to get GPILSM was described by H.P. Rang (1964). The ileum was stretched gently over a glass rod of about 0.6 - 0.7 cm in diameter and the attached mesentery was cut away. Using a blunt scalpel blade, two shallow incisions were made on ether side of the mesenteric attachment line, just cutting through the longitudinal muscle which is the outer smooth muscle layer. The GPILSM was then tangentially brushed away from the underlying tissue with a cotton-tipped applicator (Q tip). The tissue was isolated as a sheet along the length of the section and finally was cut into small sections, 1.5 to 2.0 cm in length.

Each end of the prepared GPILSM was tied by a piece of thread. One thread was fixed at the bottom of a tissue bath of 10 ml capacity and the other end was attached to a force-displacement transducer (Model FT03; Grass). The transducer was coupled to a polygraph machine (Model 7D; Grass), by which isometric tension responses of the experiments were recorded. Before the recording, the signals had been magnified several times by pre-amplifiers (Model 7P1G; Grass) and driver amplifiers (Model 7DAG; Grass) in the polygraph. The bath was filled with HPSS and maintained at 37°C by a water circulator (Model E52; Haake or Model Thermomix II; B. Braun) and aerated with 100% O_2. An initial tension of about 0.50 g was applied to each tissue. Tissues were left to equilibrate for about 45 minutes with changes of HPSS every fifteen minutes before the experiments were carried out. The initial tension of the tissue

invariably declined during the equilibration period and was readjusted to 0.25 g at the beginning of the experiment. During this period of time, the chart speed was set at 0.5 cm/min in order to observe the tension changes of the tissue. During the experiment, the recording chart speed was 12.5 cm/min. The tonic contraction of the GPILSM was the experimental parameter used to assess the activity of the compounds.

3.4.1 Experimental protocols for VJ-37 antagonism on GPILSM

The following procedure was used to obtain a non-cumulative antagonist DRC of VJ-37:

1. Antagonism of both carbachol and KCl induced contractions in GPILSM were investigated. When carbachol was used as agonist to initiate contraction, a final concentration of 2 X 10^{-7} M carbachol was added in the tissue bath. When KCl was used as agonist to initiate contraction, a stock solution of 142.9 mM isotonic KCl was used. To obtain a final concentration of 25 mM KCl in the tissue bath, 1.4 ml of HPSS was removed from the tissue bath and then was replaced with an equal volume of the isotonic KCl in this experiment.
2. After an equilibration period, a maximal dose of agonist was given and the response was monitored until a maximal tonic contraction was obtained.
3. The tissue was washed once with HPSS and after 15 minutes, step 2 was repeated.

4. Steps 2 and 3 were repeated until consistent responses were obtained, usually two to three times. These responses were treated as the maximal control response, against which the action of VJ-37 was compared. The antagonistic effect of VJ-37 was calculated as a per cent decrease of the subsequent responses relative to control response.
5. 5 minutes after the last wash in step 4, a small dose of VJ-37 was added.
6. After another 10 minutes, step 2 was repeated.
7. Once the tissue recovered to the control level, steps 5 and 6 were repeated, with a larger dose of VJ-37.
8. Steps 4 to 7 were repeated until an ID_{50} was obtained.

3.4.2 Experimental protocols for investigating the mechanism of VJ-37 antagonism on GPILSM

The following procedures were used to obtain cumulative DRCs and to elucidate the mechanism of action of VJ-37 in GPILSM. The protocols were similar to that of the guinea pig right atrial experiment:

1. After an equilibration period, a cumulative DRC to the agonist was obtained. The drugs were added in a geometrical fashion in half-log units. The maximal response of the tissue to each dose of agonist was allowed to develop and plateau (about 1.5 min/dose) prior to administration of the next higher dose.

2. The tissue was washed twice with HPSS and permitted to re-equilibrate for 15 minutes, until the the recording baseline was stable.
3. The tissue was then washed once and a dose of VJ-37, which was predetermined to be less than the ID_{50}, was added. 15 minutes later, another cumulative DRC of agonist was obtained.
4. The tissue was washed three times and allowed to re-equilibration for 15 minutes. A challenge maximal dose of agonist was then given to see whether the tissue recovered from the previous antagonist treatment.
5. The tissue was then washed twice and another dose of VJ-37, which was above the ID_{50}, was added. 15 minutes later, the third cumulative DRC of agonist was obtained.

The antagonism of VJ-37 on Ca^{2+}-induced response was obtained as follows: After an equilibration in the normal HPSS, several control KCl depolarizing responses were obtained. The bathing solution was replaced with Ca^{2+}-free HPSS (3 washes) and a final concentration of 25 mM KCl was then added. Afterwards, the tissues were re-equilibrated for 30 minutes. Finally, the above steps 1 - 5 were followed to finish $CaCl_2$ DRCs in the Ca^{2+}-free HPSS.

3.5 *In vivo* gastric acid secretory experiment using the pylorus-ligated rat model

Methods for measuring spontaneous gastric acid secretion in the rat have been described by Shay and co-workers (1954). Sprague Dawley rats (Charles River Canada Inc., St. Constant, Quebec), weighing between 160 - 270 g were chosen for the study. Each animal was housed singly in a cage with a raised base of wide wire mesh to insure immediate passage of faeces from the cage. Special care was taken to keep the cages clean at all times. The animals were starved for 48 hours and water was permitted ad libitum. At the end of 48 hours starvation, the rats were weighed again and this weight was used to calculate the rate of secretion and acid output per 100 g body weight.

Under light ether anaesthesia, the abdomen of a rat was shaved and a midline incision was made extending downward from the xiphoid for about 2 cm. The duodenum was identified and the junction between the pylorus and the duodenum was picked up gently with a curved probe. The stomach itself was not disturbed. The pylorus was ligated by a silk thread. The abdominal wall was then closed with an interrupted suture, cleaned thoroughly with physiological saline, and covered with a solution of collodion, to prevent the contamination of the gastric contents from licking the wound.

Following pyloric ligation, the test drug, VJ-37 or cimetidine or the solvent DMSO, was injected subcutaneously. The animal was placed in its cage and received no food and water during the

remainder of the experiment. One hour later, gastric acid secretion was stimulated by subcutaneous injection of histamine or solvent saline. This procedure was repeated another hour. The concentration of injected drugs were: histamine 20 mg/kg (Bickel et al., 1986); cimetidine 25 mg/kg (Katz et al., 1986); VJ-37 35.3 mg/kg. The amount of VJ-37 used was equimolar to 25 mg/kg of cimetidine. The drugs were administered at a volume of 0.1 ml/100 g body weight.

Four hours after the operation, the animal was again anaesthetized with ether, the abdomen was opened and a ligation was placed around the esophagocardiac junction. The esophagus, the duodenum and the appropriate peritoneal ligaments were clipped. The stomach was removed and washed in physiological saline. The saline attached to the stomach was soaked up by paper towels. An opening was then made along the greater curvature and the gastric juice was drained into a graduated centrifuge tube through a funnel.

The gastric contents of each stomach were analysed individually. The specimen were centrifuged (Model Sorvall GLC-2B; Du Pont Instruments) at a rotative centrifugal force of 695 g for 10 minutes. The volumes of supernatant and solids were recorded. If the volume of the solids was over 0.6 ml, the animal was discarded because this was considered to indicate possible contamination of the gastric contents by food residue or feces. The pH value of the supernatant was recorded with a pH meter (Model 109; Corning). The supernatant was transferred to a beaker and the tube was washed twice with 14.0 - 19.5 ml double distilled water, obtaining the total measuring volume of 20 ml. The supernatant was titrated to pH

7 with 0.1 M NaOH. The acid concentration and total acid output were then calculated.

3.6 Analysis of data

For all *in vitro* experiments, a minimum of three determinations were used to calculate the arithmetic mean and the standard error of the mean, which represented the average of the tissue responses and their distribution about the mean respectively. The ED_{50} and ID_{50} values of the different compounds were obtained by: 1) Using Cricket Graph, a Mcintosh computer program to draw the different excitatory and inhibitory DRCs. 2) Linear regression analysis of the responses to two to three doses of antagonists, which provided inhibition between 25 - 75% of the maximal tissue response and were within the linear portion of the inhibitory DRC, were analysed using Lotus 123, an IBM computer program to determine the ED_{50} and ID_{50} values.

Comparison of the ID_{50} values was performed using Fisher's L.S.D. (least significant difference test) after Analysis of Variance (ANOVA) was done. This test compared the ID_{50} values with each other, once a significance in the group was determined by ANOVA. For the *in vivo* rat gastric acid secretion experiment, Wilcoxon's Signed Rank Test was used to compare the data because the group size was small, the variation of data was large, and the data was not always in the normal distribution range. The calculations were completed using the SPSS/PC+, a IBM computer program and StatWorks, a Macintosh computer program. The level for significance was determined at the 5% level of significance, (i.e. $P < 0.05$).

Comparison of the compounds was based on the structural modifications made to the structure of the lead compound and the relative potencies of the derivatives. Relative potency within a structurally related group or subgroup was compared by assigning the least potent compound a value of one and determining the potency of each compound relative to this value.

CHAPTER IX

RESULTS

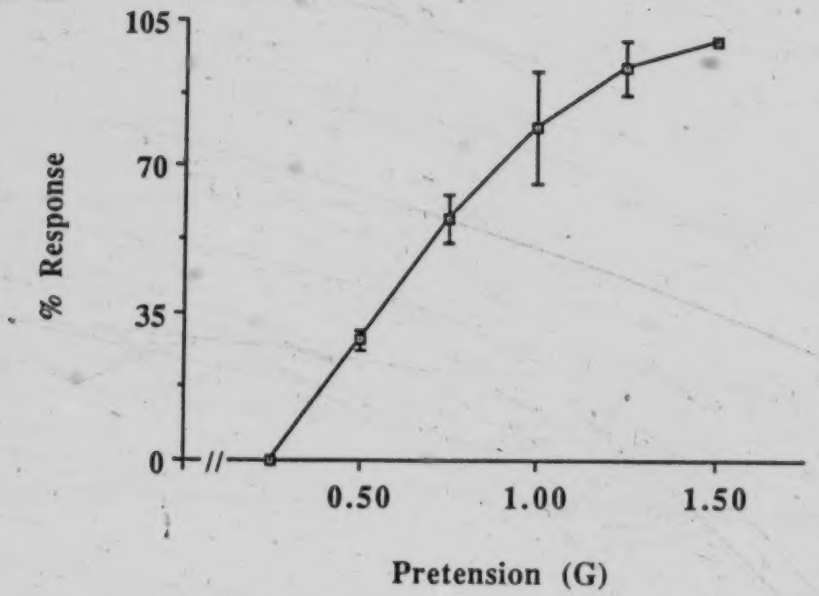
4.1 *In vitro* guinea pig right atrial experiments

4.1.1 Some control experiments

4.1.1.1 Determination of resting tension for each tissue

After every guinea pig right atrium was set up in the tissue bath, a pre-tension-contraction relationship and a pre-tension-chronotropic relationship were determined for the purpose of choosing the best resting tension for each tissue (figure 4.1). It was shown that there was a positive correlation between the pre-tension and the force of contraction within 1.25 - 1.50 g pre-tension range (figure 4.1a). The force of contraction increased about two times within this range. The maximal contractions were obtained at about 1.25 - 1.50 g tension and in the following experiments, 1.00 - 1.25 g was chosen as the resting tension. This was the 75 - 85% of the maximal pretension. In contrast, it was also demonstrated that pre-tension did not affect heart rate (figure 4.1b); so changes of the heart rate were not dependent on the resting tension of the tissue.

4.1a:



4.1b:

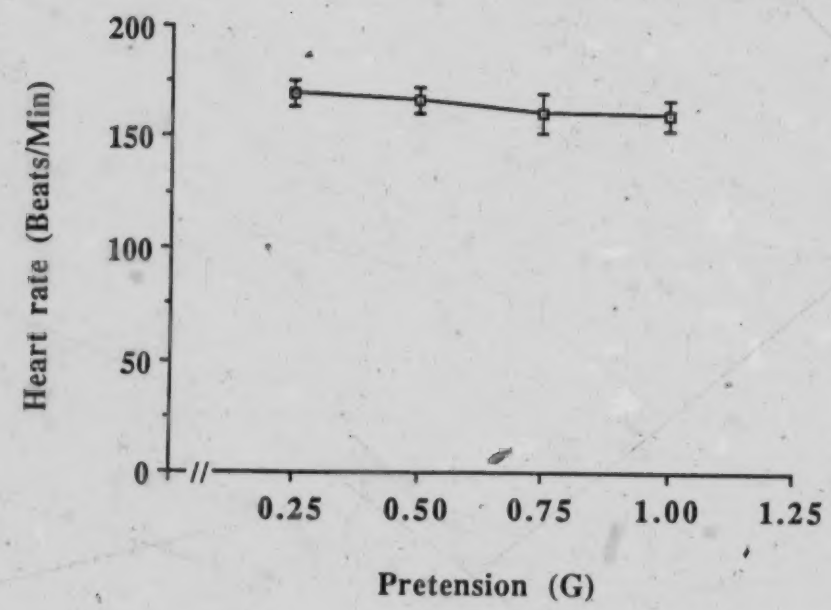


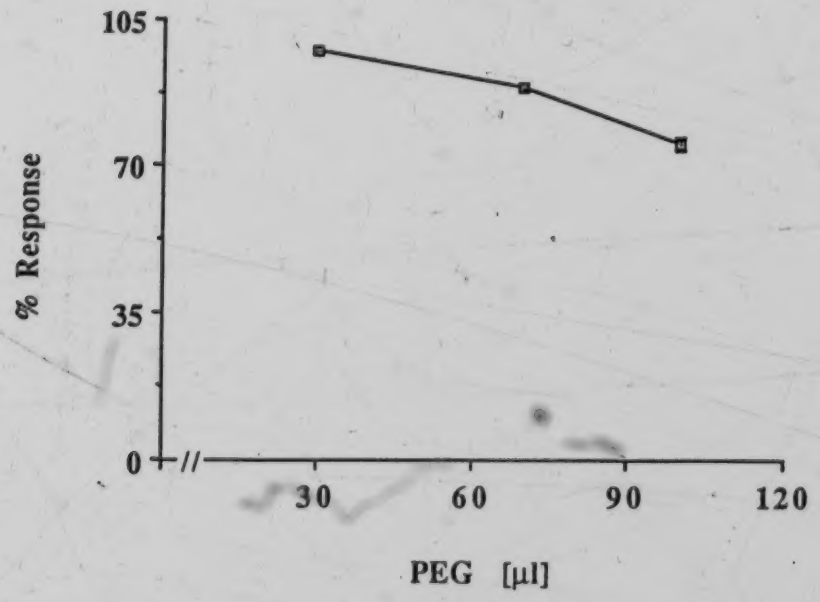
Figure 4.1 Pretension-tissue function relationship of guinea pig right atria. Values are means ± S.E.M. (standard error of the means; N = 3).

4.1a: Pretension-force of contraction relationship.
4.1b: Pretension-heart rate (HR) relationship.

4.1.1.2 Solvent evaluation for drugs which could not be dissolved in double distilled water or HPSS

Before the formal screening experiment, several control experiments were carried out in order to examine any possible influence of other substances on the experiments and to establish the standard screening procedures. The first control experiment involved the determination of an appropriate solvent for drugs which could not be dissolved in double distilled water or HPSS. One solvent chosen was PEG. Figure 4.2a shows that PEG decreased the maximal chronotropic response to histamine in guinea pig right atria. In the presence of 30 µl of PEG in the tissue bath (with a final concentration of 5.4×10^{-3} M PEG, the highest concentration of the solvent to be used in the experiments), the DRC of histamine was depressed significantly (figure 4.2b). PEG was therefore not used as the solvent in our experiments. The second solvent selected was DMSO. Figure 4.3a shows that DMSO did not affect the maximal chronotropic response to histamine in the concentrations tested. In the presence of 30 ul of DMSO in the organ bath (with a final concentration of 7.8×10^{-3} M, the highest concentration of the solvent to be employed), the DRC of histamine did not change significantly (figure 4.3b). This showed that within the dose range selected, DMSO did not affect the tissue response. DMSO was thus used as the solvent for drugs which could not be dissolved in double distilled water or HPSS in subsequent experiments.

4.2a:



4.2b:

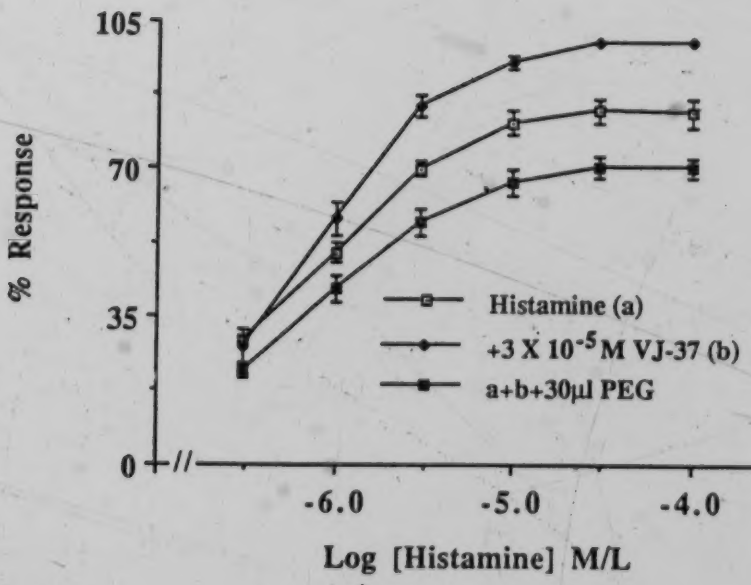
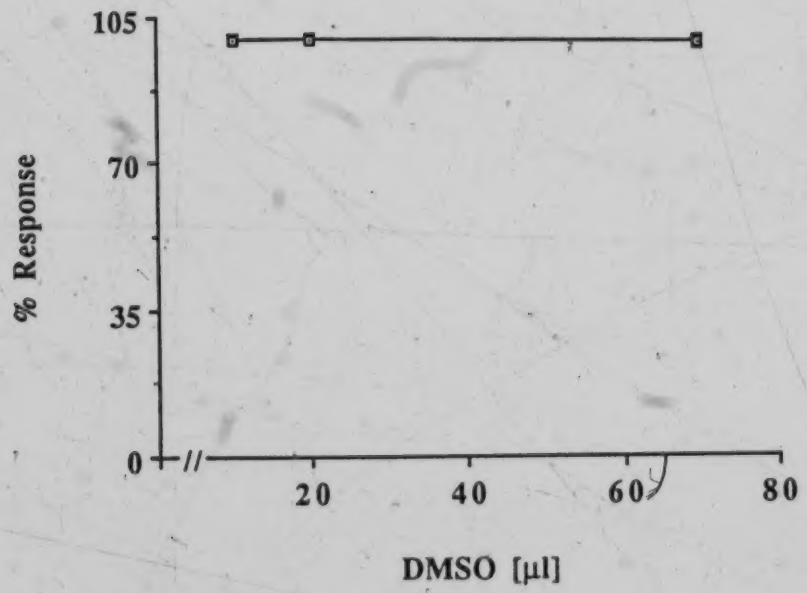


Figure 4.2 Antagonism on histamine-induced chronotropic response in guinea pig right atria by PEG. Values are means ± S.E.M. (N = 3).

4.2a: Inhibitory DRC of PEG on the maximal tissue response to histamine (3.0×10^{-5} M).

4.2b: Antagonism of PEG (30 µl, —■—) on histamine response.

4.3a:



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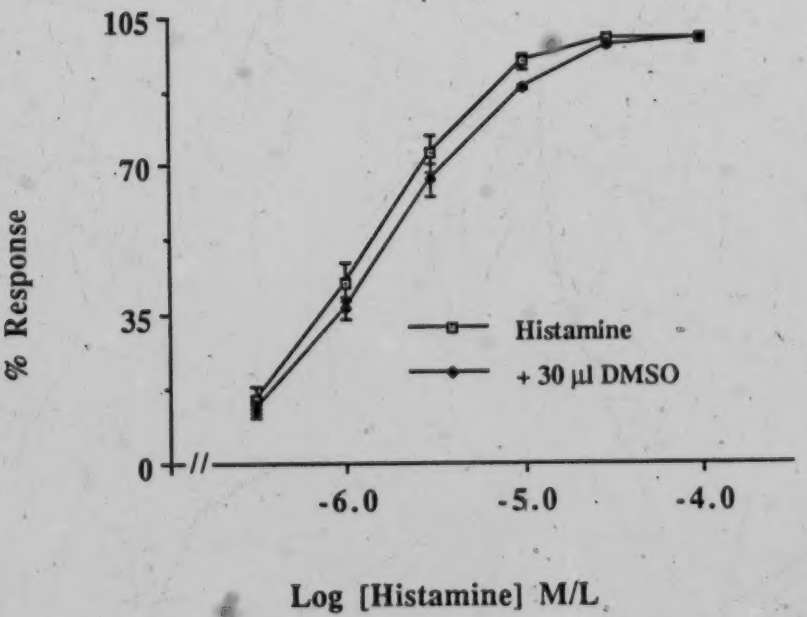


Figure 4.3 Effect of DMSO on histamine-induced chronotropic response in guinea pig right atria. Values are means ± S.E.M.

4.3a: Effects of DMSO on the maximal tissue response to histamine (3.0×10^{-5} M).

4.3b: Effect of DMSO (30 µl/10 ml, —●—) on histamine response.

4.1.1.3 Consideration of the possible interference of the sympathetic catecholamine release in drug screening

It is almost certain that in guinea pig right atrium, the histamine mediated-positive chronotropic effect can be solely attributed to histamine H_2-receptor activation and not to catecholamine release (Wolff and Levi, 1986). However, in order to insure that catecholamine release is not involved, β-receptor antagonists are often added in the histamine H_2-receptor antagonist screening procedure (Parsons et al., 1977; Ossama et al., 1985).

In order to rule out the possible influence of catecholamine in the H_2-receptor screening, the effect of the β-receptor antagonist nadolol was investigated. Figure 4.4 shows the inhibitory DRC of nadolol on the maximal dose (3.0×10^{-7} M) of isoproterenol in guinea pig right atria. More than 85% of the isoproterenol-induced chronotropic response was blocked by 3.0×10^{-5} M of nadolol which indicated that this concentration of nadolol should prevent any catecholamine-induced effects. Subsequently, in considering the effect of nadolol on the histamine-induced response, it was found that nadolol had no significant effect on the maximal dose (3.0×10^{-5} M) of histamine at a concentration of 3×10^{-5} M (figure 4.5a). In the presence of 1.0×10^{-5} M or 3.0×10^{-5} M of nadolol, the DRCs of histamine were not affected at all (figure 4.5b). In agreement with Wolff and Levi's hypothesis, these present results indicate that the release of catecholamines had no role in

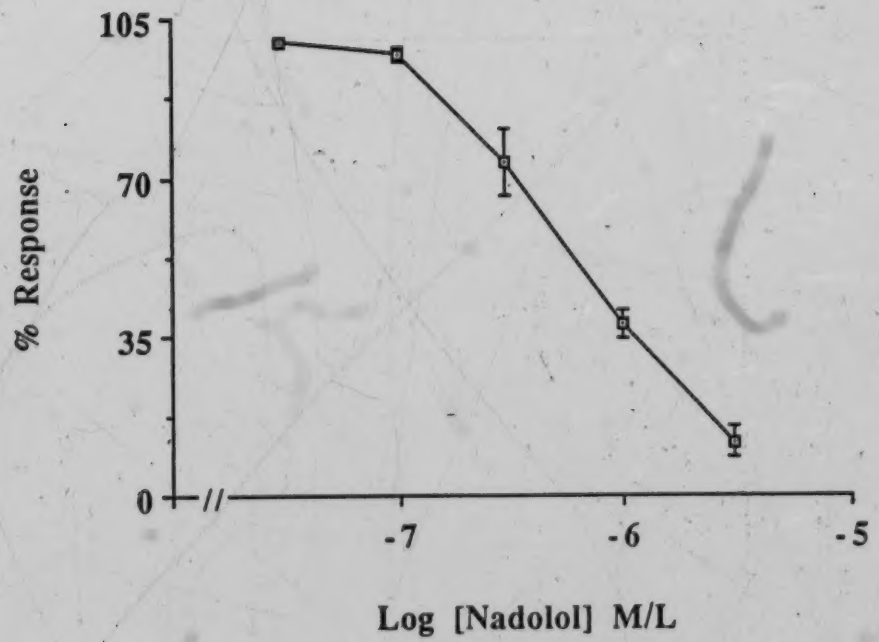
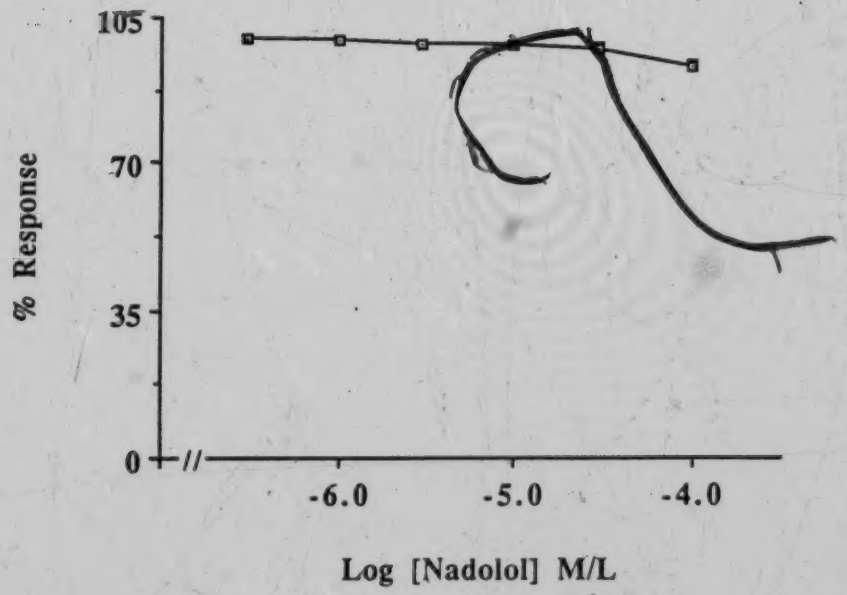


Figure 4.4 Antagonism on isoproterenol-induced (3.0×10^{-7} M) chronotropic response in guinea pig right atria by nadolol. Values are means ± S.E.M. (N = 3).

4.5a:



4.5b:

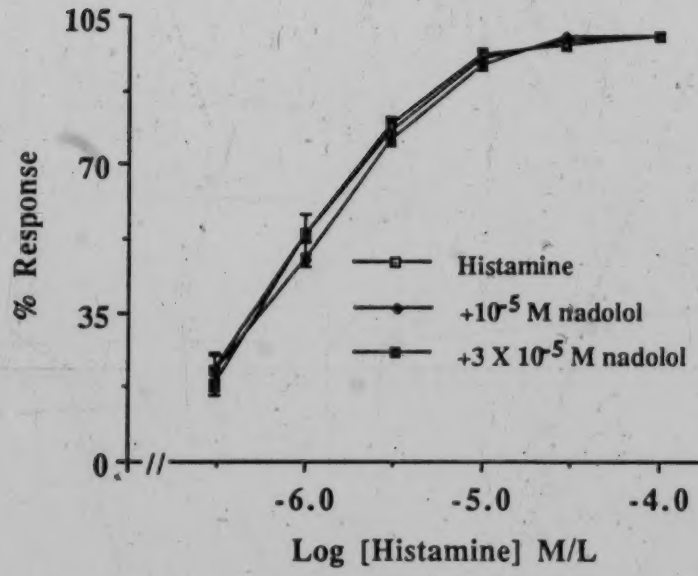


Figure 4.5 Effect of nadolol on histamine-induced chronotropic response in guinea pig right atria. Values are means ± S.E.M. (N = 3).

4.5a: Inhibitory DRC of nadolol on the maximal dose of histamine (3.0×10^{-5} M).

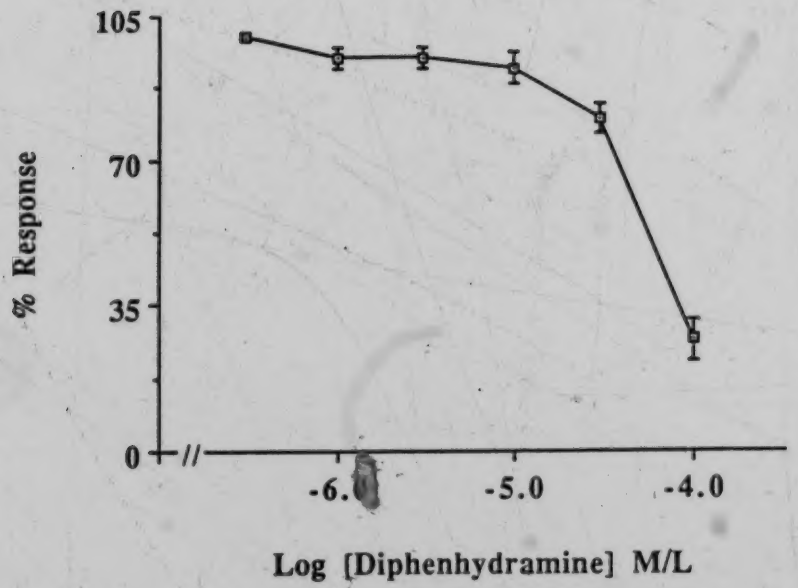
4.5b: Effect of nadolol (10^{-5} M, —●—; 3×10^{-5} M, —■—) on histamine response.

the positive chronotropic effect of histamine in guinea pig right atrium. Nevertheless, 1.0 X 10^{-6} M nadolol, which blocked more than 60% of the isoproterenol-induced chronotropic effect, was added into the tissue bath 15 minutes before the addition of histamine in all histamine H_2-receptor antagonist screening experiments in this thesis, in order to insure that only histamine H_2-receptor mediated responses were being considered.

4.1.1.4 Differentiation of histamine receptors on guinea pig right atrium

It has been well established that histamine H_2-receptors predominate in guinea pig right atrium (Black et al., 1972; McNeill, 1984). This was reconfirmed in our trials. Figure 4.6 shows the effects of a histamine H_1-receptor antagonist (diphenhydramine) on guinea pig right atrium. It was observed that diphenhydramine failed to antagonize the histamine-induced chronotropic effect in guinea pig right atria up to a concentration of 1.0 X 10^{-5} M (figure 4.6a). Pretreatment with 1.0 X 10^{-5} M and 3.0 X 10^{-5} M diphenhydramine did not lead to a significant shift of the DRC of histamine to right. In contrast to this, the histamine-induced chronotropic effect was greatly affected by cimetidine, a histamine H_2-receptor antagonist (figure 4.7). Cimetidine, in the dose range between 1.0 X 10^{-6} M to 1.0 X 10^{-4} M, inhibited the maximal response to histamine (3.0 X 10^{-5} M) in a dose related manner in the same animal model (figure 4.7a). The ID_{50} of cimetidine was 1.87 $\pm$ 0.36 X 10^{-5} M. The pA_2 value calculated

4.6a:



4.6b:

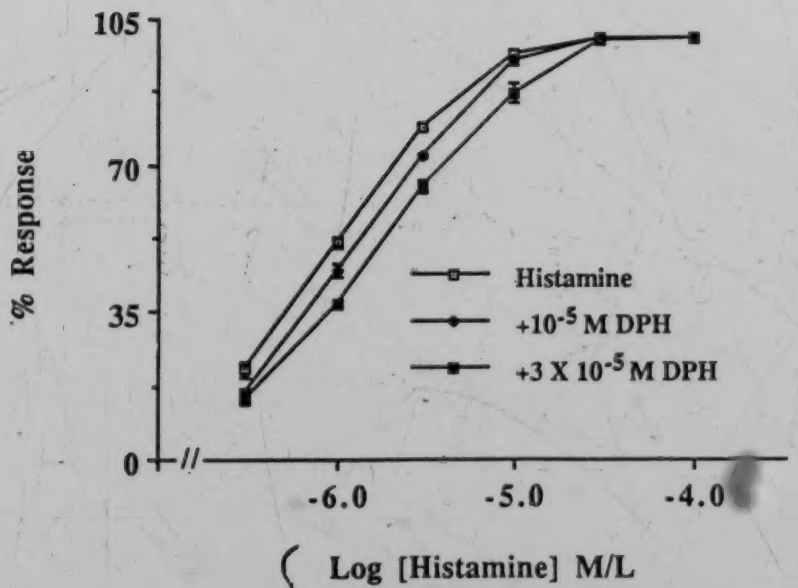
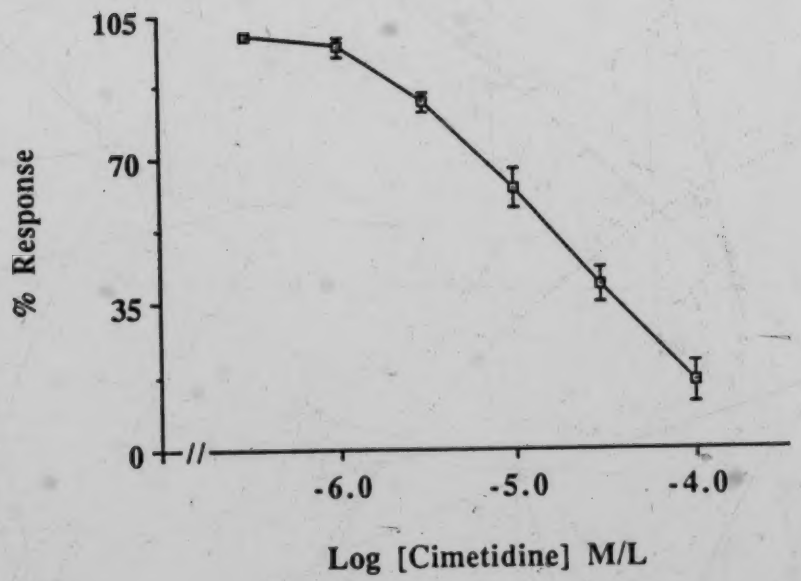


Figure 4.6 Effect of diphenhydramine (DPH) on histamine-induced chronotropic response in guinea pig right atria. Values are means ± S.E.M. (N = 3).

4.6a: Inhibitory DRC of diphenhydramine on the maximal tissue response to histamine (3.0×10^{-5} M).

4.6b: Effect of diphenhydramine (10^{-5} M, —●—; 3×10^{-5} M, —■—) on histamine response.

4.7a:



4.7b;

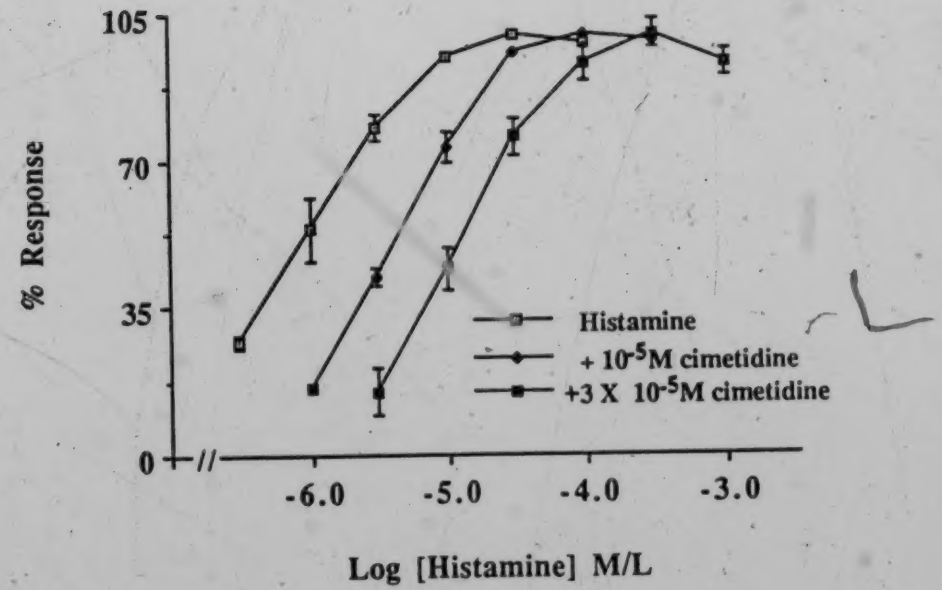


Figure 4.7 Antagonism on histamine-induced chronotropic response in guinea pig right atria by cimetidine. Values are means ± S.E.M. (N = 3).

4.7a: Inhibitory DRC of cimetidine on the maximal tissue response to histamine (3.0×10^{-5} M).

4.7b: Antagonism of cimetidine (10^{-5} M, —●—; 3.0×10^{-5} M, —■—) on histamine response.

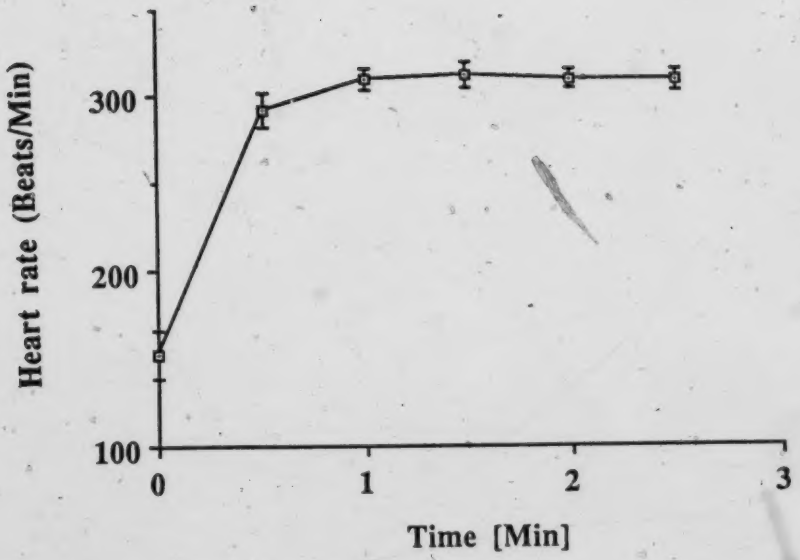
from the data was 5.3. In the presence of 1.0 X 10^{-5} M and 3.0 X 10^{-5} M cimetidine, the DRCs of histamine were shifted to right in a parallel fashion, without depression of the maximal response (figure 4.7b). These data, therefore, support the conclusion that in guinea pig right atrium, the histamine response is exclusively due to histamine H_2-receptor stimulation.

4.1.1.5 Determination of time intervals for drug addition

In order to determine the optimal time intervals for histamine (agonist) addition, the time course of tissue response to histamine was analysed (figure 4.8). After the addition of histamine, the maximal response was obtained within 1.0 minute (figure 4.8a); after 6 X 10^{-5} M VJ-37 pretreatment and then the addition of histamine, 1.5 - 2.0 minutes was required to obtain the maximal response (figure 4.8b). Thus, in this thesis, the time interval for measuring the histamine response without antagonist pretreatment was kept at 2.0 minutes; the time interval for measuring the histamine response after antagonist pretreatment was 3.0 minutes.

The time course of tissue response to antagonist was found to be different in atria which had not been exposed to histamine (agonist) than in atria which had been exposed to histamine. Figure 4.9a indicated that after histamine pretreatment for 10.0 minutes, about 4 minutes was needed for a dose of 7 X 10^{-5} M VJ-37 to establish the maximal inhibition. On the other hand, without histamine pretreatment, about 28 minutes was required for a dose of 6 X 10^{-5} M VJ-37 to attain maximal inhibition (figure 4.9b). Thus, in this

4.8a:



4.8b:

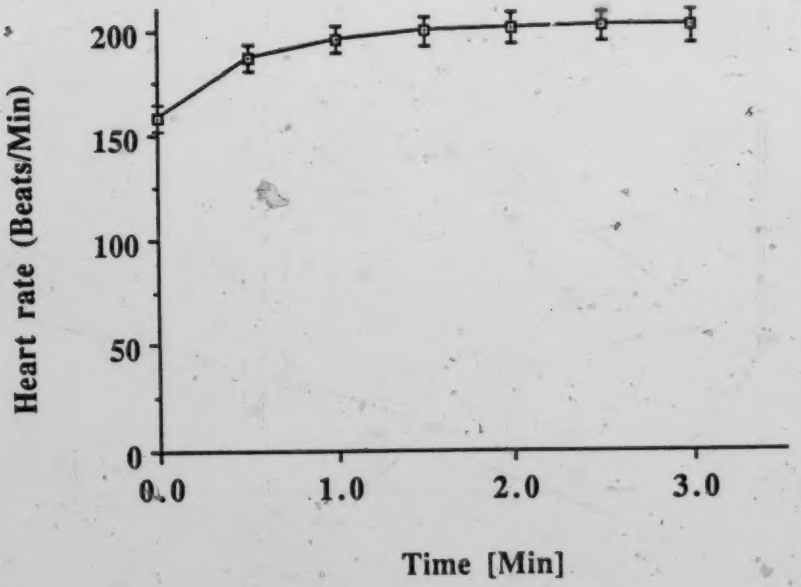
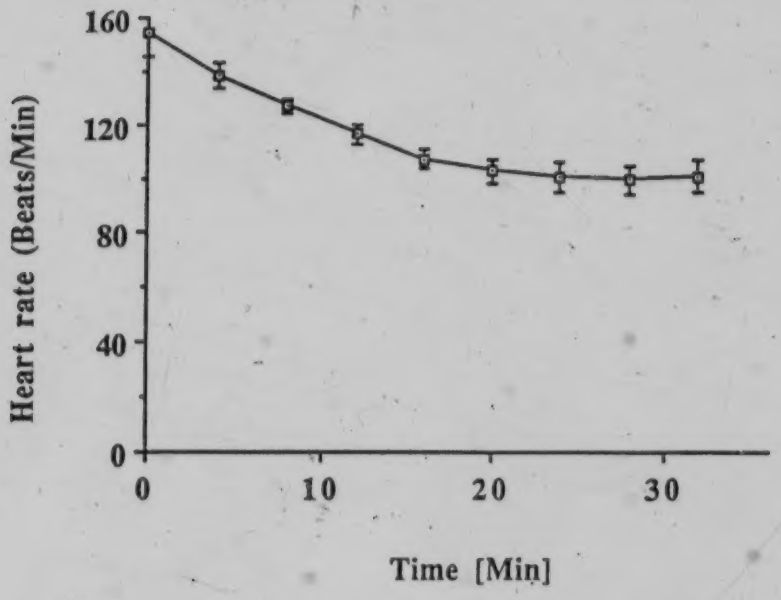


Figure 4.8 Time course of tissue response to histamine in guinea pig right atria before and after VJ-37 pretreatment.

4.8a: Before VJ-37 pretreatment, time course of tissue response to a dose of histamine (3.0×10^{-5} M).

4.8b: After VJ-37 (6×10^{-5} M) pretreatment for 10 minutes, time course of tissue response to a dose of histamine (2×10^{-6} M).

4.9a:



4.9b:

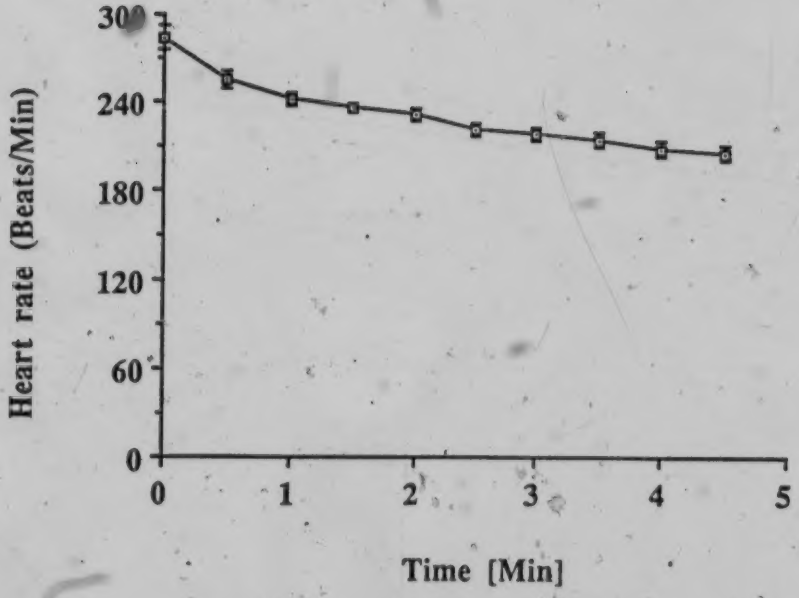


Figure 4.9 Time course of tissue response to VJ-37 in guinea pig right atria before and after histamine pretreatment.

4.9a: Before histamine pretreatment, time course of tissue response to a dose of VJ-37 (6.0×10^{-5} M).

4.9a: After histamine (3.0×10^{-5} M) pretreatment for 10 minutes, time course of tissue response to a dose of VJ-37 (7.0×10^{-5} M).

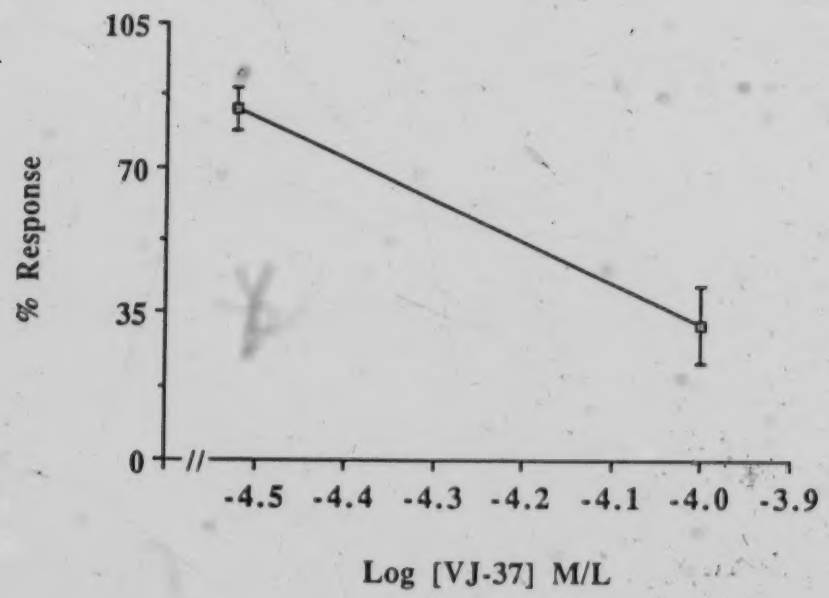
thesis, the time interval for antagonist additions or antagonist DRC determination in the presence of maximal dose of histamine was kept at 4.0 minutes; without histamine pretreatment, the time interval after adding a dose of antagonist and beginning another histamine DRC was 30.0 minutes.

4.1.2 Pharmacological characteristics of the novel synthesized compounds screened

The classical pharmacological characteristics of all the tested compounds tend to be similar and are typically represented by the results of VJ-37. Figure 4.10 shows the antagonistic effect of VJ-37 on the histamine-increased heart rate in guinea pig right atrium. VJ-37 inhibited the elevated heart rate in a dose related manner (figure 4.10a). At the same time, it also decreased the force of contraction of the tissue about 20% (data not shown). Furthermore, after pretreatment with 3×10^{-5} M and 6×10^{-5} M VJ-37 for 30 minutes, the histamine-induced positive chronotropic DRCs were shifted to the right in a non-parallel manner, with obvious depression of the maximal response (figure 4.10b). The effect was reversible since VJ-37 could be washed out. The results indicate that VJ-37 noncompetitively inhibits the histamine H_2-receptor-induced positive chronotropic response in guinea pig right atrium.

All compounds screened were found to be noncompetitive antagonists for the histamine H_2-receptor mediated positive chronotropic response. They all could be washed out by two to three

4.10a:



4.10b:

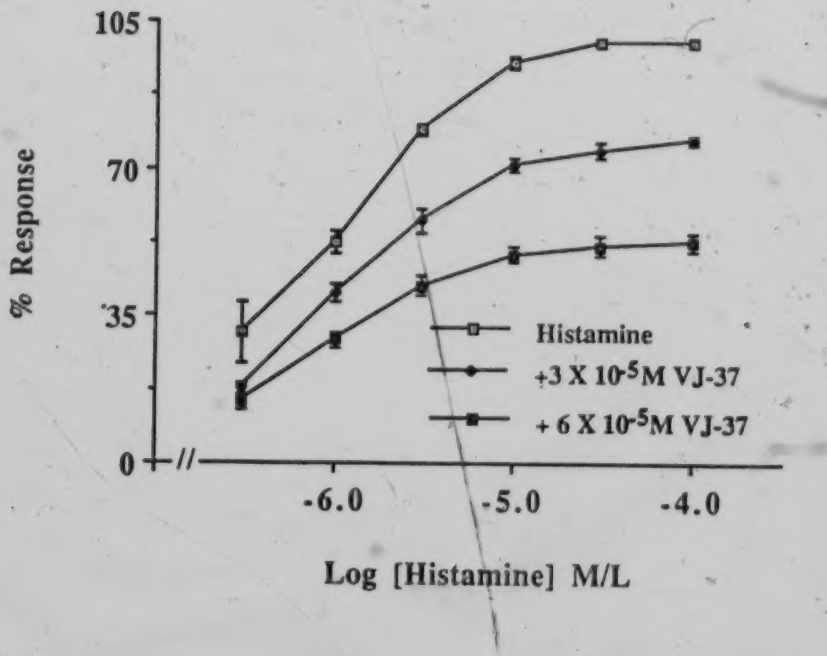


Figure 4.10 Antagonism on histamine-induced chronotropic response in guinea pig right atria by VJ-37. Values are means ± S.E.M. (N = 3).

4.10a: Inhibitory DRC of VJ-37 on the maximal dose of histamine (3.0×10^{-5} M).

4.10b: Antagonism of VJ-37 (3.0×10^{-5} M, —●—; 6.0×10^{-5} M, —■—) on histamine response.

rinses with HPSS solution. The majority of the compounds tested decreased the resting heart rate and the force of contraction of the tissues simultaneously. In summary, they all behaved as noncompetitive antagonists of the histamine H_2-receptor in guinea pig right atrium.

4.1.3 Structure-activity relationship analysis of novel synthesized compounds

The structures of the novel synthesized compounds are all related to histamine H_2-receptor antagonists such as cimetidine or ranitidine which contain a flexible connecting chain,. Such compounds possesses three fundamental structural features, including a substituted aromatic heterocyclic (Het) ring and a polar H-bonding group or an "urea equivalent (Y)", joined together by a connecting chain in the general structure: Het-$CH_2SCH_2CH_2$-Y (Ganellin, 1985; Agrawal et al., 1988). The antagonist activities of novel compounds with different substituted Het rings and "urea equivalent Y"s were compared using the guinea pig right atrial assay.

4.1.3.1 Antagonist activities of compounds possessing a pyridyl Het moiety and substitutions at the R and X positions of the 1,4-dihydropyridyl "urea equivalent Y"

A group of compounds with a pyridyl Het moiety and substitutions at the R and X positions of the 1,4-dihydropyridyl "urea equivalent Y" were investigated to determine the effect of 1) a pyridyl ring

system and its point of attachment at Het end of the connecting chain; 2) incorporation of the terminal amino group into a 1,4-dihydropyridyl ring system at Y; and 3) the C-4 dihydropyridyl R substituent at Y system.

Table 4.1 shows the relative potencies (RP) and ID_{50} values of the novel analogues within this group. Relative to cimetidine, these compounds were not very potent. The most potent compound, VJ-11, with a RP of 26.5, was about half as potent as that of cimetidine. The least potent compound in the group, VJ-22, was about fifty-eight times less potent than that of cimetidine and was about 27 times less potent than that of VJ-11.

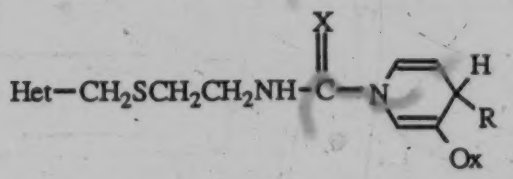
The results demonstrate that the point of the Het end attachment of the pyridyl ring has an effect on the antagonistic activity when the remaining structures are similar. The 2-pyridyl analogues were more active than that of 3-pyridyl and 4-pyridyl derivatives (Table 4.1; figure 4.11 and 4.12). The relative order of activity was 2-pyridyl > 3 pyridyl > 4 pyridyl.

The data also suggests that the R substitution at the C-4 position of the 1,4-dihydropyridyl ring is a determinant of the antagonist activity. Table 4.1 and figure 4.11 also show that the relative potency order of the R substituent was, in most cases, n-Bu > Ph > Me.

The data does not indicate that selecting either the 2-cyano-1,1-iminodiamino (X=NCN) or the 2-nitro-1,1-ethenediamino ($X=CHNO_2$) moiety at the X position of the 1,4-dihydropyridyl ring can affect the antagonist activity of the compounds. Table 4.1 and figure 4.12 show that in most cases (except for 4-pyridyl Het

Table 4.1

Antagonist Activities of Compounds Possessing a Pyridyl Het Moiety and Substitutions at the R and X Positions of the 1,4-Dihydropyridyl "Urea Equivalent Y"



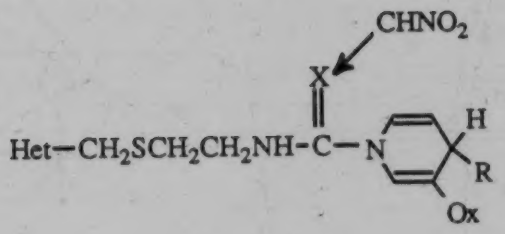
Compound	Het	R	X	ID_{50} ± SEM (M)	RP
VJ-24	2-pyridyl	Me	NCN	$8.77 \pm 1.84 \times 10^{-5}$	12.4
VJ-22	3-pyridyl	Me	NCN	$1.09 \pm 0.88 \times 10^{-3}$*	1.0
VJ-23	4-pyridyl	Me	NCN	$1.84 \pm 0.32 \times 10^{-4}$	5.9
VJ-12	2-pyridyl	n-Bu	NCN	$7.73 \pm 2.31 \times 10^{-5}$	14.1
VJ-10	3-pyridyl	n-Bu	NCN	$7.60 \pm 1.09 \times 10^{-5}$	14.3
VJ-11	4-pyridyl	n-Bu	NCN	$4.12 \pm 0.09 \times 10^{-5}$	26
VJ-15	2-pyridyl	Ph	NCN	$8.12 \pm 0.44 \times 10^{-5}$	13.4
VJ-13	3-pyridyl	Ph	NCN	$1.36 \pm 0.03 \times 10^{-4}$	8.0
VJ-14	4-pyridyl	Ph	NCN	$1.06 \pm 0.02 \times 10^{-4}$	10.3
VJ-18	2-pyridyl	n-Bu	$CHNO_2$	$9.43 \pm 0.61 \times 10^{-5}$	11.6
VJ-19	3-pyridyl	n-Bu	$CHNO_2$	$9.34 \pm 2.16 \times 10^{-5}$	11.7
VJ-20	4-pyridyl	n-Bu	$CHNO_2$	$1.35 \pm 0.24 \times 10^{-4}$	8.1
VJ-21	2-pyridyl	Ph	$CHNO_2$	$7.45 \pm 1.40 \times 10^{-5}$	14.6
VJ-16	3-pyridyl	Ph	$CHNO_2$	$1.33 \pm 0.10 \times 10^{-4}$	8.2
VJ-17	4-pyridyl	Ph	$CHNO_2$	$2.15 \pm 0.77 \times 10^{-4}$	5.1
Cimetidine				$1.87 \pm 0.36 \times 10^{-5}$	58.3

Contd..

Table 4.1 Contd..

ID_{50} is the required dose to obtain 50% inhibition of the maximal tissue response. Values are means ± S.E.M. (N=3). RP is the Relative Potency in comparison to the weakest compounds in the series.

* Compare to all other groups, $P < 0.05$.



Het = 2-, 3-, or 4-pyridyl
R = n-Bu or Ph

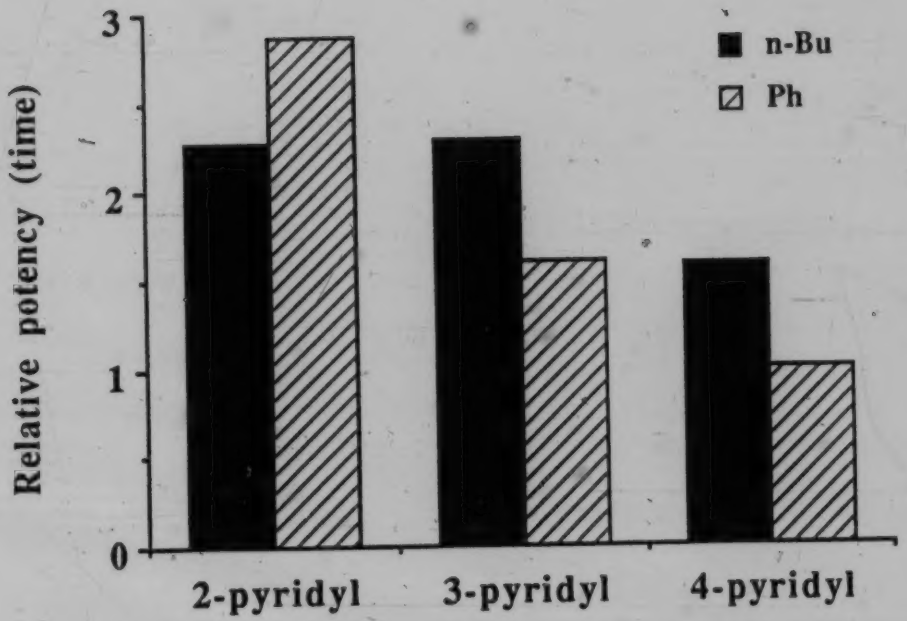
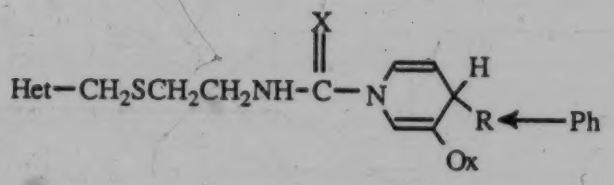


Figure 4.11 Relative potencies of compounds possessing a pyridyl Het moiety and substitutions at the R and 2-amino-1,1-ethenediamino ($CHNO_2$) substituted-X positions of the 1,4-dihydropyridyl "urea equivalent Y".



Het = 2-, 3-, or 4-pyridyl
X = NCN or $CHNO_2$

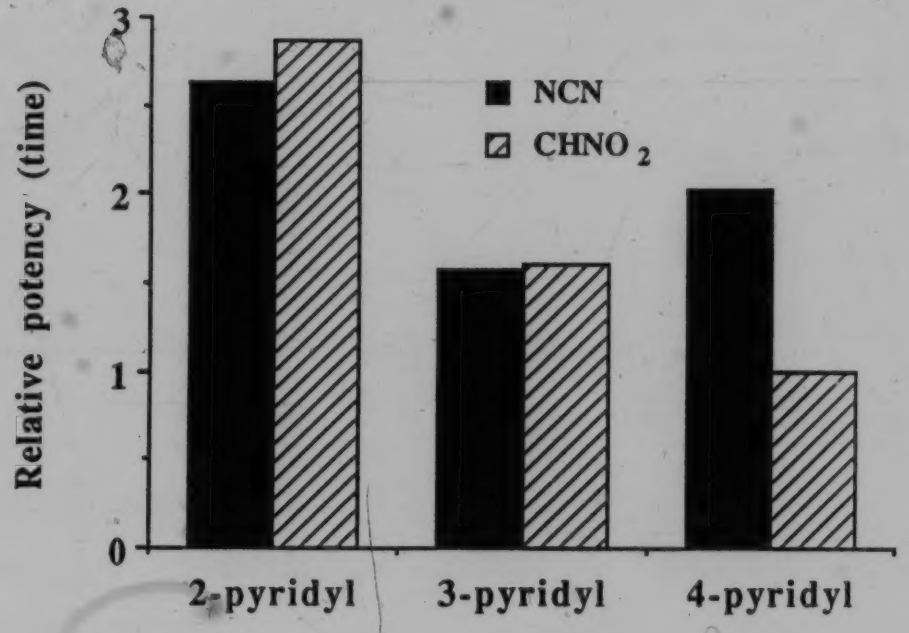


Figure 4.12 Relative potencies of compounds possessing a pyridyl Het moiety and substitutions at the X and phenyl substituted-R positions of the 1,4-dihydropyridyl "urea equivalent Y".

substituents), there was little difference in activity between these two groups.

4.1.3.2 Antagonist activities of compounds possessing a pyridyl or a 4-methyl-5-imidazolinyl Het moiety and a substitution at the R position of the 2-amino-3-nitropyridyl "urea equivalent Y"

A group of compounds with a pyridyl or a 4-methyl-5-imidazolinyl Het moiety and a substitution at the R position of the 2-amino-3-nitropyridyl "urea equivalent Y" were examined for to determine the effect of 1) the Het ring system and its point of attachment; and 2) the R-substituent at the Y system.

Table 4.2 shows the RP and ID_{50} values of novel analogues of this group. Overall, these compounds were a slightly less potent when compared with compounds in Table 4.1. The most potent compound, VJ-37, with a RP of 8.1, was about one-quarter as potent as cimetidine. The least potent compound VJ-38 was approximately thirty-one times less potent than cimetidine and was about eight times less potent than VJ-37.

These results indicate that the point of attachment of the pyridyl substituent at the Het position affects the antagonist activities. Table 4.2 and figure 4.13. show that the 4-pyridyl analogues were more active than the 3- and 2-pyridyl isomers. The R-substituent present on the 2-amino-3-nitropyridyl ring system was a determinant of the antagonist activity. Table 4.2 and figure 4.13 also show that the relative potency order of the R substituent

Table 4.2

Antagonist Activities of Compounds Possessing a Pyridyl or a 4-Methyl-5-imidazolinyl Het Moiety and a Substitution at the R Position of the 2-Amino-3-nitropyridyl "Urea Equivalent Y"

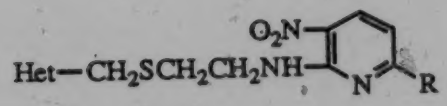
Compounds	Het	R	ID_{50} ± SEM (M)	RP
VJ-28	2-pyridyl	H	$2.14 \pm 0.48 \times 10^{-4}$	2.7
VJ-26	4-pyridyl	H	$1.11 \pm 0.19 \times 10^{-4}$	5.1
VJ-31	5-Me-4-im	H	$9.23 \pm 2.13 \times 10^{-5}$	6.2
VJ-35	4-pyridyl	Cl	$3.77 \pm 1.15 \times 10^{-4}$*	1.5
VJ-34	5-Me-4-im	Cl	$5.33 \pm 1.57 \times 10^{-4}$**	1.1
VJ-36	2-pyridyl	OMe	$1.08 \pm 0.16 \times 10^{-4}$	5.3
VJ-37	4-pyridyl	OMe	$7.05 \pm 1.17 \times 10^{-5}$	8.1
VJ-38	2-pyridyl	NH_2	$5.71 \pm 1.60 \times 10^{-4}$**	1.0
VJ-30	3-pyridyl	NH_2	$5.68 \pm 0.64 \times 10^{-4}$**	1.0
VJ-39	4-pyridyl	NH_2	$3.34 \pm 0.49 \times 10^{-4}$***	1.7
Cimetidine			$1.87 \pm 0.36 \times 10^{-5}$	30.5

ID_{50} is the required dose to obtain 50% inhibition of the maximal tissue response. Values are mean ± S.E.M. (N=3). RP is the Relative Potency in comparison to the weakest compounds in the series. 4-Me-5-im = 4-methyl-5-imidazolinyl.

* Compare to VJ-26, VJ-31, VJ-36, or VJ-37, $P < 0.05$.

** Compare to VJ-28, VJ-26, VJ-31, VJ-36, or VJ-37, $P < 0.05$.

*** Compare to VJ-37, $P < 0.05$.



Het = 2-, 3-, or 4-pyridyl
R = H, Cl, OMe, or NH_2

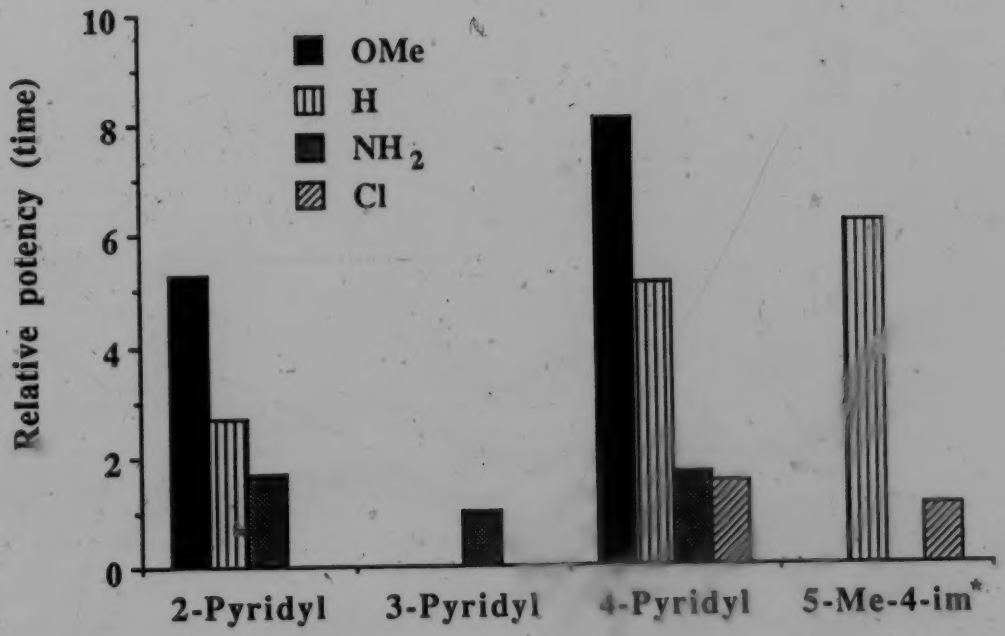


Figure 4.13 Relative potencies of compounds possessing a pyridyl Het or a 4-methyl-5-imidazolinyl moiety and a substitution at the R position of the 2-amino-3-nitropyridyl "urea equivalent Y".
* 4-Me-5-im = 4-Methyl-5-imidazolinyl.

generally was OMe > H > NH_2 > Cl. Moreover, the differences in activity exhibited by a 4-pyridyl or 4-methyl-5-imidazolinyl Het-substitution were small.

4.1.3.3 Antagonist activity of compounds possessing a pyridyl Het moiety and a substitution at the X position of the 3-aminopyridyl or a 2-amino-3-nitro-1H-pyridyl-6-one "urea equivalent Y"

Several compounds with a pyridyl Het moiety and a substitution at the X position of the 3-aminopyridyl or a 2-amino-3-nitro-1H-pyridyl-6-one "urea equivalent Y" were examined to determine the effect of 1) the Het ring system and its point of attachment; and 2) incorporation of miscellaneous acyclic "urea equivalent Y" into the heterocyclic ring system.

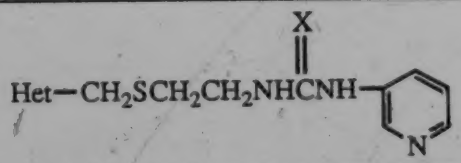
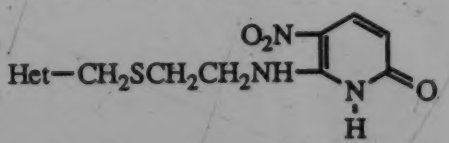
A general decrease in activity was produced by the modifications made in this series of analogues. Table 4.3 shows that all of the structural modifications resulted in a dramatic reduction in the antagonist activity with very similar ID_{50}s. The compounds in this group were more than two orders of magnitude less potent than that of cimetidine.

4.1.4 Mechanism of action of VJ-37 antagonism in guinea pig right atrium

Since all the screened novel compounds noncompetitively antagonized histamine-induced positive chronotropic responses,

Table 4.3

Antagonist Activities of Compounds Possessing a Pyridyl Het Moiety and a Substitution at the X Position of the 3-Aminopyridyl or the 2-Amino-3-nitro-1H-pyridyl-6-one "Urea Equivalent Y"

Compounds	Het	X	ID_{50} ± SEM (M)	RP
				
VJ-25	2-pyridyl	NCN	$1.46 \pm 0.97 \times 10^{-3}$	2.1
VJ-27	4-pyridyl	NCN	$1.17 \pm 0.08 \times 10^{-3}$	2.6
VJ-33	2-pyridyl	$CHNO_2$	$1.97 \pm 0.99 \times 10^{-3}$	1.6
				
VJ-29	2-pyridyl		$2.03 \pm 0.62 \times 10^{-3}$	1.5
VJ-32	4-pyridyl		$3.09 \pm 0.24 \times 10^{-3}$	1.0
Cimetidine			$1.87 \pm 0.36 \times 10^{-5}$	165.0

ID_{50} is the required dose to obtain 50% inhibition of the maximal tissue response. Values are means ± S.E.M. (N=3). RP is the Relative Potency in comparison to the weakest compounds in the series.

further experiments were carried out to see whether these compounds could also antagonize the effect of other chronotropes in the guinea pig right atrium. After one experiment, a simple test was completed to see whether VJ-24 could antagonize isoproterenol, another positive chronotrope.

The antagonism of VJ-24 on both the histamine- and the isoproterenol-induced positive chronotropic effect is presented in figure 4.14. Guinea pig heart rates were increased by both isoproterenol (3×10^{-7} M) and histamine (5×10^{-5} M). After stimulation with histamine, the heart rates were decreased by VJ-24 (3.0×10^{-5} M). This inhibition by VJ-24 could not be overcome by another maximal dose of isoproterenol (3×10^{-7} M). Since VJ-24 could antagonize not only the histamine-induced chronotropism but also isoproterenol-mediated chronotropism, this data supports the concept that VJ-24 does not act specifically via the histamine H_2-receptor.

In order to explore the possible mechanism of action of these compounds, one of the most potent compounds, VJ-37, was further evaluated. The effects of VJ-37 on β_1-adrenergic receptors, muscarinic receptors, Ca^{2+} channels, intracellular cAMP, as well as sarcoplasmic reticulum (SR) mediated mechanisms were therefore investigated.

All the compounds employed, isoproterenol, Bay K 8644, forskolin, 8-bromo-cAMP, milrinone and caffeine but not atropine, had a dose related positive chronotropic effect in the guinea pig right atria. Table 4.4 shows the stimulatory potencies (ED_{50}) of the different positive chronotropes and the inhibitory potencies

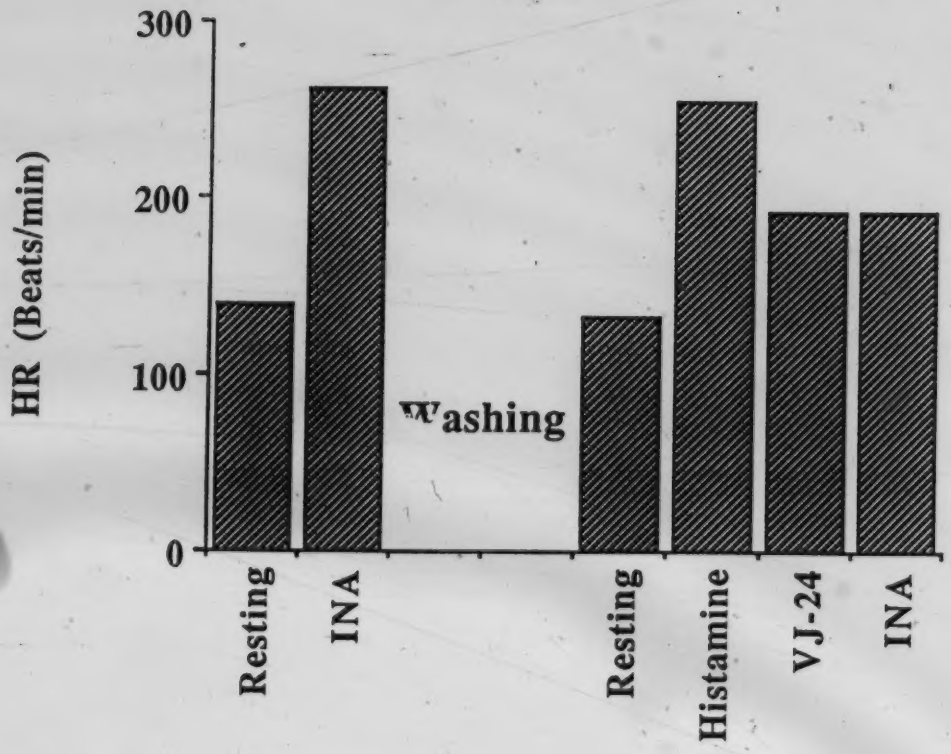


Figure 4.14 Antagonism on either the isoproterenol (INA)- or the histamine-induced chronotropic response in guinea pig right atrium by VJ-24. N = 1.

Table 4.4

Antagonisms of VJ-37 on Agonist-induced Chronotropic Responses in Guinea Pig Right Atria

Agonist	ED_{50} of Agonist ± SEM (M)	ID_{50} of VJ-37 ± SEM (M)
Isoproterenol	$5.44 \pm 0.33 \times 10^{-9}$	$5.45 \pm 0.55 \times 10^{-5}$
Bay K 8644	$2.25 \pm 0.63 \times 10^{-7}$	$4.24 \pm 0.09 \times 10^{-5}$
Forskolin	$2.26 \pm 0.89 \times 10^{-7}$	$4.79 \pm 0.66 \times 10^{-5}$
8-Bromo-cAMP	$3.85 \pm 0.24 \times 10^{-4}$	$4.34 \pm 0.08 \times 10^{-5}$
Milrinone	$2.24 \pm 0.61 \times 10^{-5}$	$6.19 \pm 0.46 \times 10^{-5}$*
Caffeine	$1.66 \pm 0.17 \times 10^{-3}$	$4.00 \pm 0.44 \times 10^{-5}$
Histamine	$9.53 \pm 0.41 \times 10^{-7}$	$7.05 \pm 1.17 \times 10^{-5}$**

ED_{50} is the required dose to obtain 50% of the maximal tissue response. ID_{50} is the required dose to obtain 50% inhibition of the maximal tissue response. Values are means ± S.E.M. (N=3).

* Compare to Bay K 8644, 8-bromo-cAMP, or caffeine, $P < 0.05$.

** Compare to isoproterenol, Bay K 8644, 8-bromo-cAMP, or caffeine, $P < 0.05$.

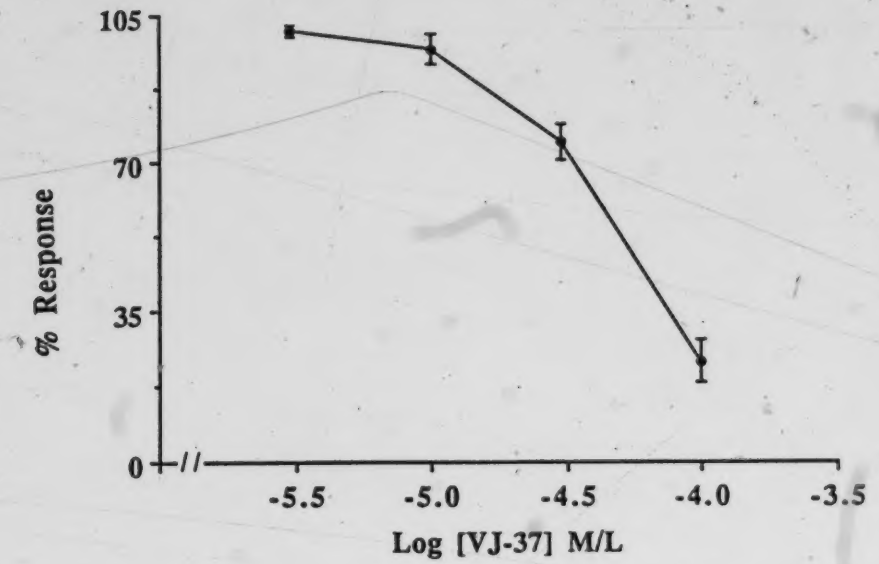
(ID_{50}) of VJ-37 on the maximal stimulatory response of these different agents. The ED_{50}s of the different positive chronotropes varied dramatically, ranging from 5.44 X 10^{-9} M (isoproterenol) up to 1.66 X 10^{-3} M (caffeine). This, in turn, reflected the different potencies of these positive chronotropes. However, the ID_{50}s of VJ-37 on the maximal response of these different positive chronotropes were similar (except milrinone), ranging from the lowest value of 6.19 X 10^{-5} M (milrinone) to the highest value of 4.00 X 10^{-5} M (caffeine). These values were significantly smaller than the value of histamine (7.05 X 10^{-5} M).

Figures 4.15 to 4.18 show that VJ-37 antagonized the chronotrope-increased heart rates. In the case of all the positive chronotropes which could be washed out, including isoproterenol, 8-bromo-cAMP, milrinone and caffeine, VJ-37 antagonized their responses noncompetitively. Bay K 8644 and forskolin could not be washed out, therefore the characteristics of the inhibition by VJ-37 could not be determined.

4.2 <u>In vitro</u> GPILSM experiment

GPILSM was used to further explore the possible actions of VJ-37 on Ca^{2+} channels. The rationale for using this tissue is based on the following: 1) GPILSM is dependent on extracellular Ca^{2+} for contraction and Ca^{2+} antagonism can be evaluated directly; and 2) GPILSM contain muscarinic receptors which when stimulated, open Ca^{2+} channels and initiate contraction.

4.15a:



4.15b:

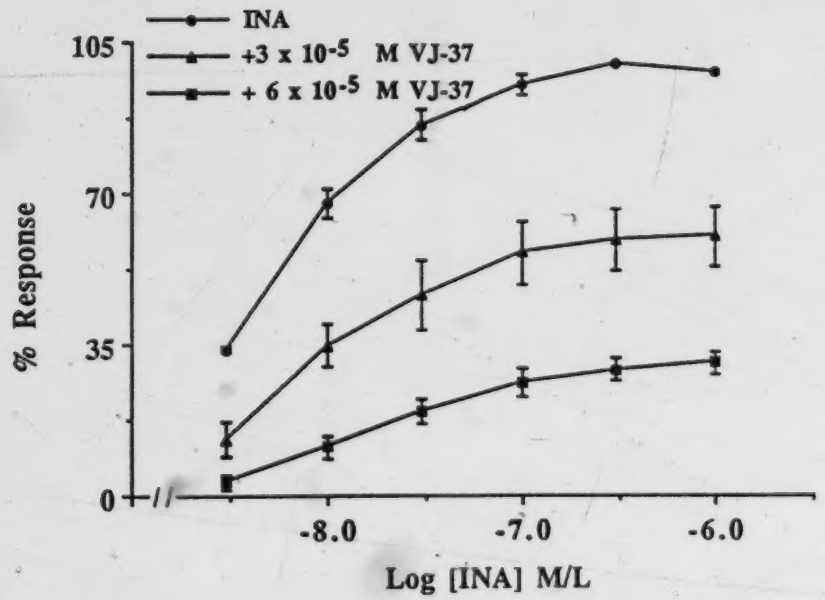
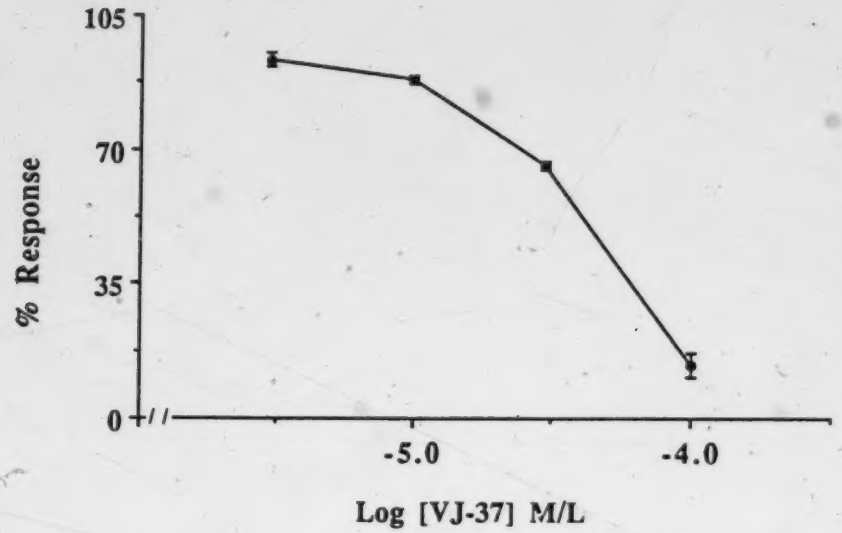


Figure 4.15 Antagonism on isoproterenol (INA)-induced chronotropic response in guinea pig right atria by VJ-37. Values are means ± S.E.M. (N = 3).

4.15a: Inhibitory DRC of VJ-37 on the maximal dose (3.0×10^{-7} M) of isoproterenol.

4.15b: Antagonism of VJ-37 on isoproterenol (3.0×10^{-5} M, —●—; 6.0×10^{-5} M, —■—) response.

4.16a:



4.16b:

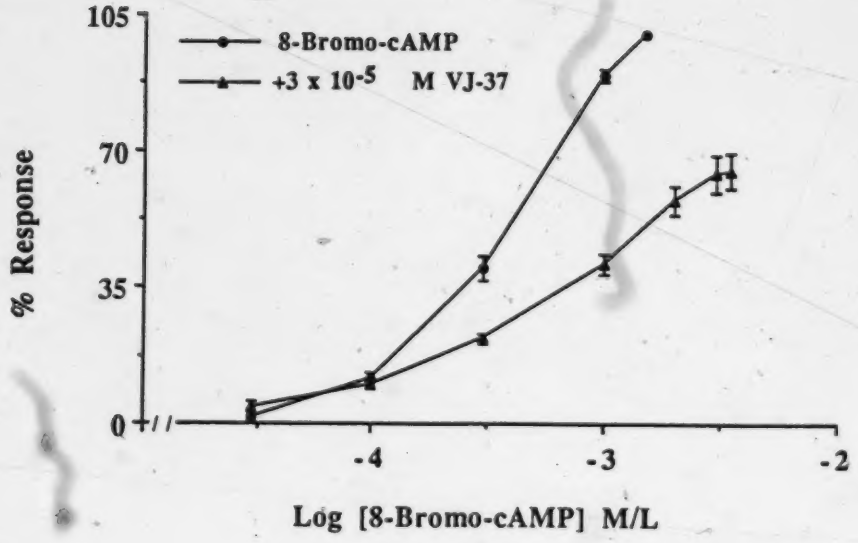
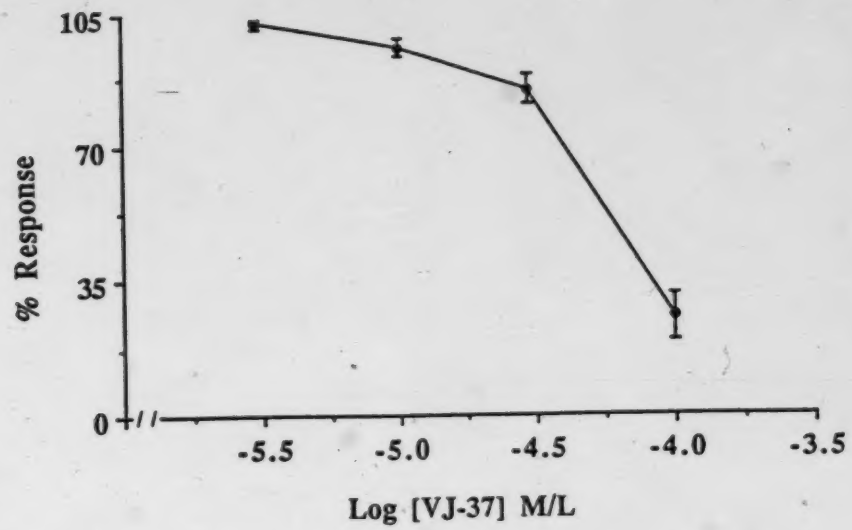


Figure 4.16 Antagonism on 8-bromo-cAMP-induced chronotropic response in guinea pig right atria by VJ-37. Values are means ± S.E.M. (N = 3).

4.16a: Inhibitory DRC of VJ-37 on the tissue response of 8-bromo-cAMP (1.5×10^{-3} M).

4.16b: Antagonism of VJ-37 (3.0×10^{-5} M, -▲-) on 8-bromo-cAMP response.

4.17a:



4.17b:

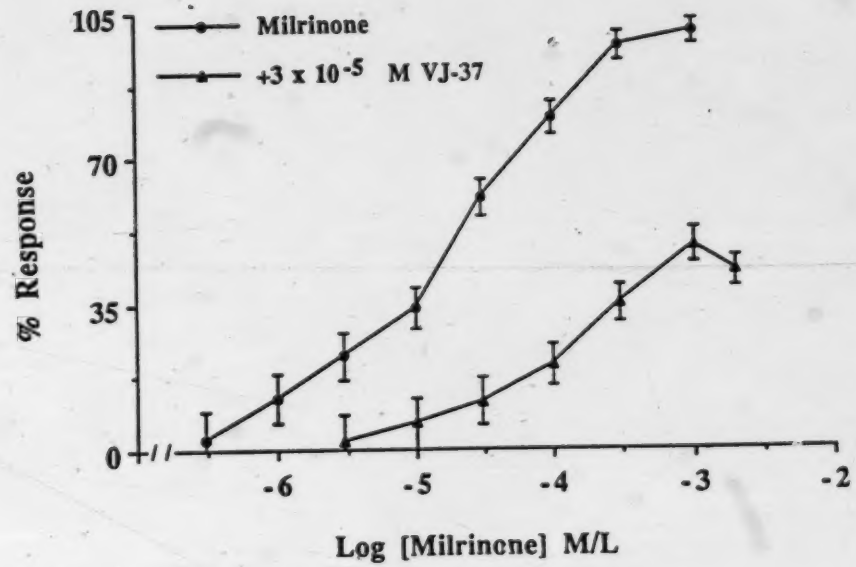
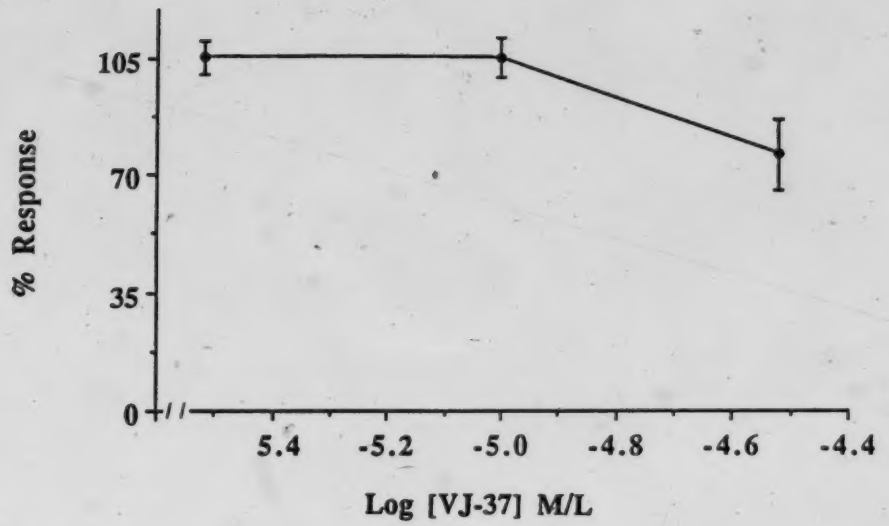


Figure 4.17 Antagonism on milrinone-induced chronotropic response in guinea pig right atria by VJ-37. Values are means ± S.E.M. (N = 3).

4.17a: Inhibitory DRC of VJ-37 on the maximal dose of milrinone (1.0×10^{-3} M).

4.17b: Antagonism of VJ-37 (3.0×10^{-5} M, -●-) on milrinone response.

4.18a:



4.18b:

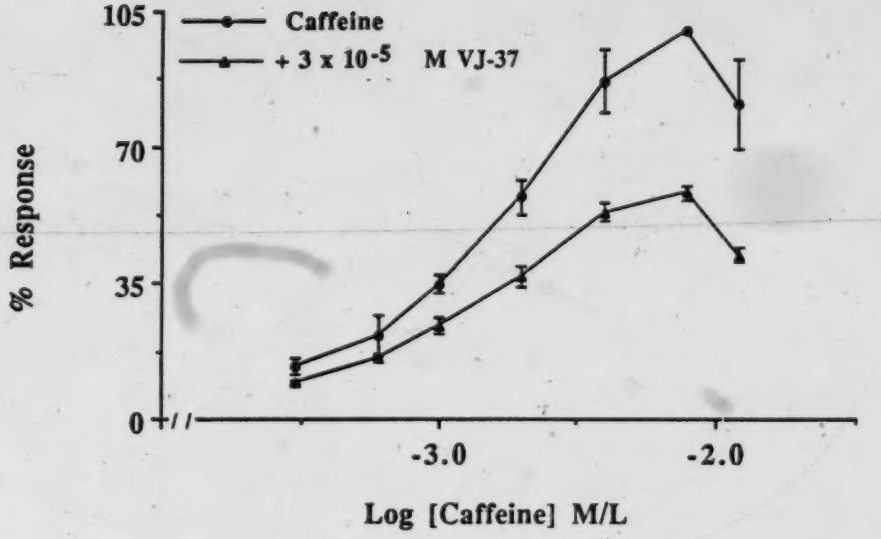


Figure 4.18 Antagonism on caffeine-induced chronotropic response in guinea pig right atria by VJ-37. Values are means ± S.E.M. (N = 3).

4.18a: Inhibitory DRC of VJ-37 on the maximal dose of caffeine (8.0×10^{-3} M).

4.18b: Antagonism of VJ-37 (3.0×10^{-5} M, —•—) on caffeine response.

VJ-37 antagonized the response to either a maximal dose of KCl, a voltage sensitive Ca^{2+} channels activator, or carbachol, a muscarinic receptor agonist, in a dose related manner. The ID_{50}s were 1.18 ± 0.11 X 10^{-5} M and 6.02 ± 0.93 X 10^{-6} M respectively, which was significantly lower than that for the antagonism of VJ-37 on histamine-induced chronotropic response in guinea pig right atrium (7.05 ± 1.17 X 10^{-5} M; $P < 0.05$). This result support a calcium channel antagonist effect may be involved.

The nature of the antagonism of VJ-37 on the DRC of $CaCl_2$ was also investigated and found to be noncompetitive. Figure 4.19 shows that the DRC of $CaCl_2$ was shifted to right in a non-parallel fashion by VJ-37, with an obvious decline of the maximal response.

The nature of antagonism of the VJ-37 on the muscarinic receptor mediated response is illustrated in figure 4.20. It was found that VJ-37 antagonized the response noncompetitively. The DRC of carbachol was shifted to right in a non-parallel fashion, with significant depression of the maximal response (figure 4.20a). In contrast with this finding, the DRC of carbachol was competitively shifted to the right by atropine, a muscarinic receptor antagonist, without depression of the maximal response (figure 4.20b).

The results suggest that VJ-37 does not directly act via inhibition muscarinic receptors, but the possibility exists that the calcium channel may be inhibited

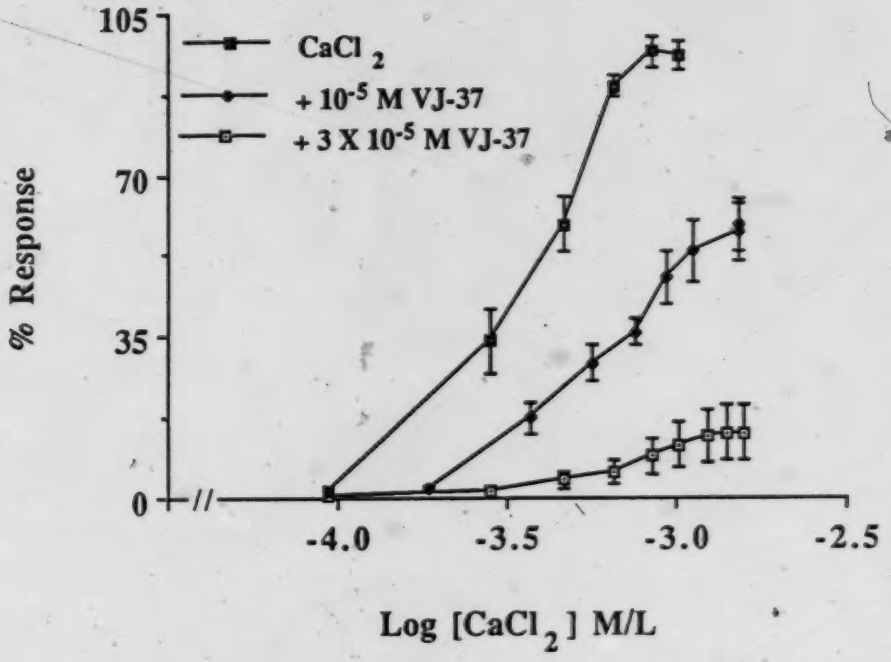
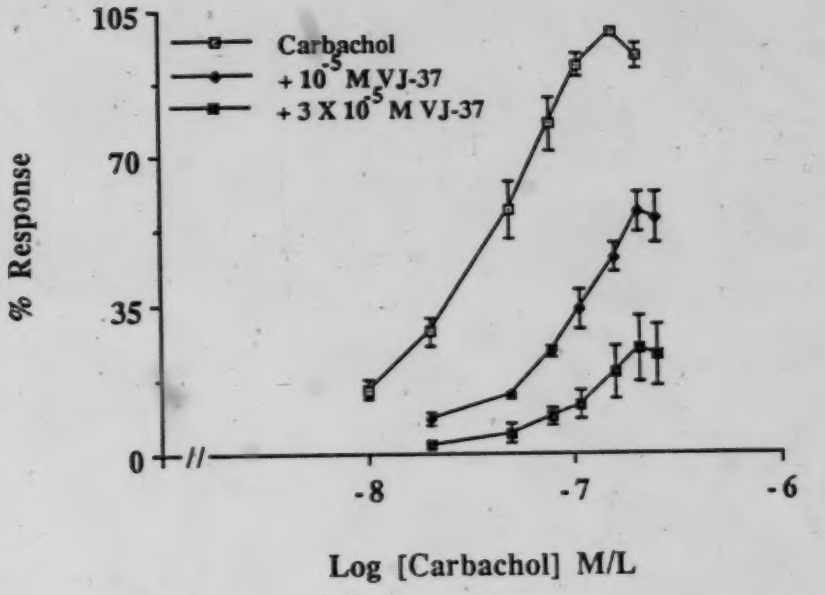


Figure 4.19 After 2.5 X 10^{-2} M KCl pretreatment, antagonism on $CaCl_2$-induced tonic contraction in GPILSM by VJ-37 (10^{-5} M, ; 3.0 X 10^{-5} M,). Values are means ± S.E.M. (N = 3).

4.20a:



4.20b:

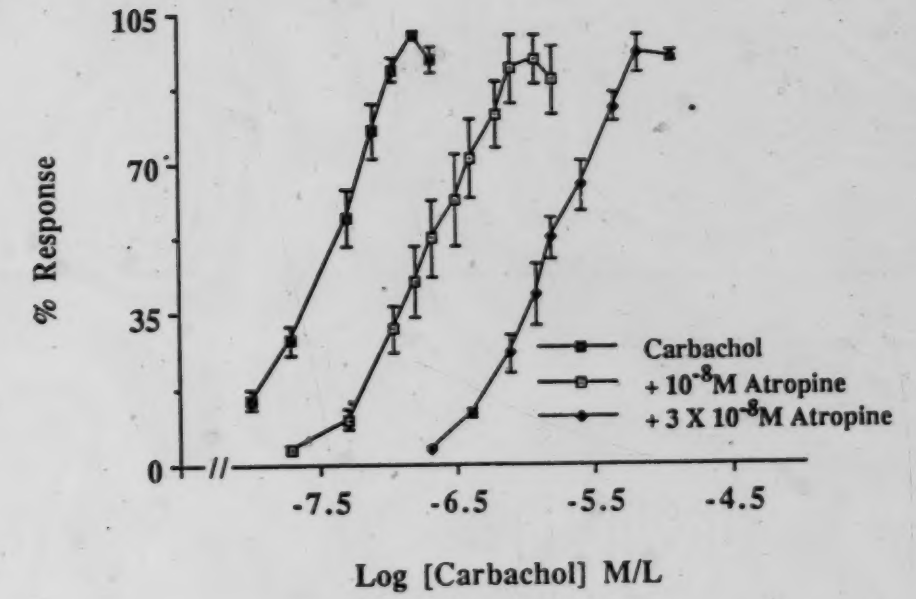


Figure 4.20 Antagonism on carbachol-induced tonic contraction in GPILSM by both VJ-37 and atropine . Values are means ± S.E.M. (N = 3).

4.20a: Antagonism of VJ-37 (10^{-5} M , —●— ; 3.0 X 10^{-5} M, —■—) on carbachol response.

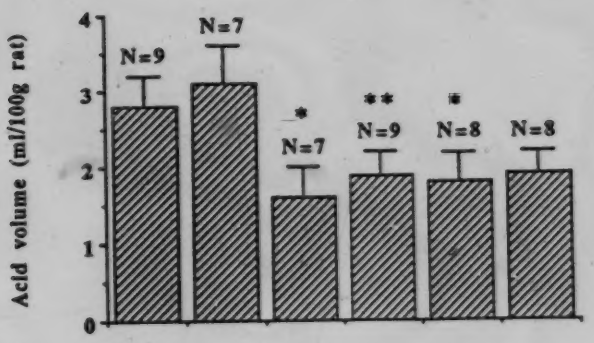
4.20b: Antagonism of atropine (10^{-8} M, —□— ; 3.0 X 10^{-8} M, —●—) on carbachol response.

4.3 *In vivo* pylorus-ligated rat gastric acid secretion experiment

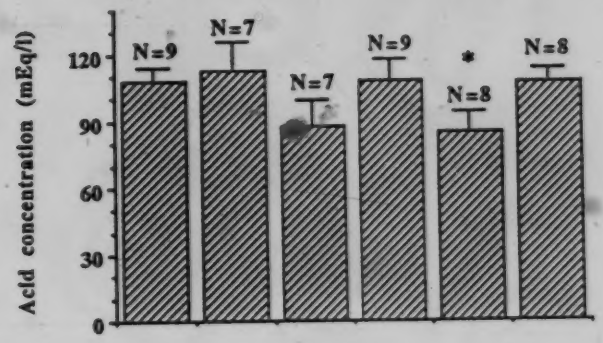
The major therapeutic application of histamine H_2-receptor antagonism is to eliminate hyper-gastric acid secretion. The experiments were carried out to evaluate the effectiveness of VJ-37 to inhibit gastric acid secretion employing the *in vivo* pylorus-ligated rat model.

VJ-37 antagonized the gastric acid secretion in the pylorus-ligated rat. Figure 4.21 shows that both VJ-37 and cimetidine equally and significantly decrease gastric acid volume (figure 4.21a), total gastric acid concentration (except cimetidine; figure 4.21b), and as a result, decreased total gastric acid output (figure 4.21c). However, the histamine-induced gastric acid secretion was not significantly different from the basal acid secretion (the standard error of the histamine-stimulated group was very large). But VJ-37 and cimetidine decreased the histamine-stimulated gastric acid volume (figure 4.21a) and total gastric acid output (figure 4.21c).

4.21a:



4.21b:



4.21c:

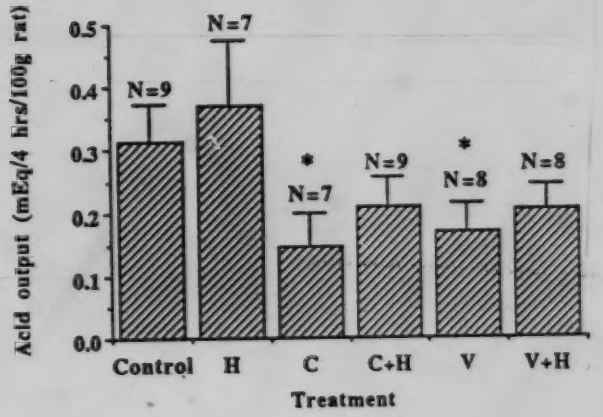


Figure 4.21 Effects of agonist and antagonists on gastric acid secretion in pylorus-ligated rat. Bars represent standard errors of the mean. Asterisks mean that values between the control and the treatment group are significantly different at $p < 0.05$. H = Histamine. C = Cimetidine. V = VJ-37.

4.21a: Effects of agonist and antagonists on gastric acid secretion.

4.21b: Effects of agonist and antagonists on gastric acid concentration.

4.21c: Effects of agonist and antagonists on total gastric acid output.

CHAPTER V

DISCUSSION

5.1 Guinea pig right atrium, a good model for histamine H_2-receptor antagonist screening

Histamine has long been recognized as a cardiac stimulant since Dale and Laidlaw demonstrated its many physiological effects on mammalian tissues in 1910 (Jones, 1966). Before the introduction of specific histamine H_2-receptor antagonists, it was recognized that the effects of histamine on isolated guinea pig heart could not be antagonized by the classical antihistamines (Trendelenburg, 1960). Later in the 1970's, Black and co-workers (1972) observed that histamine increased the sinoatrial rate of the isolated guinea pig right atrium and this stimulation was selectively antagonized by the histamine H_2-receptor antagonist burimamide. This formed the basis of one of three important techniques for the identification and quantitative evaluation of compounds which act at histamine H_2-receptors. The three techniques are the *in vitro* guinea pig atrium assay, the *in vitro* rat uterus assay and the *in vivo* rat gastric acid secretion assay (Black et al., 1972).

It has been well established that the only histamine receptor in guinea pig right atrium is the H_2-receptor (Black et al., 1972; Levi et al., 1982; McNeill, 1984; Wolff and Levi, 1986). In

agreement with other authors (McNeill, 1984; Wolff and Levi, 1986), the data in this thesis showed that the histamine H_1-receptor antagonist diphenhydramine did not affect the DRC of histamine in the guinea pig right atrium whereas the histamine H_2-receptor antagonist cimetidine competitively shifted the DRC of histamine to the right. The pA_2 value of cimetidine was 5.3, which was consistent with the values reported in the literature (Black et al., 1972; Levi et al., 1982). The present data supported the hypothesis that the only histamine receptor in guinea pig right atrium was the H_2-receptor, which makes this tissue very useful for histamine H_2-receptor antagonist screening. Others have also found that guinea pig cardiac responses to histamine were quite similar to that of primates and man (Levi et al., 1979); and that there was a high concentration of histamine in the guinea pig right atrium, making it a sensitive tissue for histamine release studies (Giotti et al., 1966; Assem et al., 1987). Furthermore, this in vitro biological assay has provided the easiest way to carry out experiments which avoid the possible influence of other histamine receptors or other aminergic systems, which can play a role in the in vivo gastric acid secretory experiment. All these advantages suggested that the guinea pig right atrium chronotropic response assay was one of the best animal tissue models for histamine H_2-receptor antagonist screening.

It was confirmed (Wolff and Levi, 1986) that in guinea pig right atrium, the histamine induced-positive chronotropic response was exclusively associated with histamine H_2-receptor activation and was not partly due to catecholamine release. However in the

conventional histamine H_2-receptor antagonist screening protocols, β-receptor antagonists were added to the tissue before the use of histamine in order to eliminate the influence of β-receptor activation by released catecholamines (Parsons et al., 1977; El-Badry et al., 1985). In order to determine the possible influence of adrenergic receptor activation on drug screening, the effect of the β-receptor antagonist nadolol on drug screening was investigated in this thesis. The results demonstrated that 3.0×10^{-5} M nadolol had almost no effect on the tissue response to a maximal dose of histamine; while the same concentration of nadolol inhibited more than 85% of the isoproterenol-induced chronotropic response. In the presence of 1.0×10^{-5} M and 3.0×10^{-5} M nadolol, the DRC of histamine was not affected at all. This definitely ruled out the possibility that the positive chronotropic effect of histamine on guinea pig right atrium was partly due to the release of catecholamines. In agreement with the present findings, Parsons and co-workers (1977) also showed that another β-receptor antagonist, propranolol, did not affect the DRC of dimaprit, a histamine H_2-receptor agonist. In view of the look of the involvement of catecholamines in the histamine-mediated response of guinea pig right atrium, β-receptor antagonists were not used in the subsequent experiments.

5.2 Structure-activity relationship analysis of histamine H_2-receptor antagonists with novel "urea equivalent Y"

The first compound to be described as a histamine H_2-receptor

antagonist was burimamide (Black et at., 1972). The discovery of this compound facilitated histamine H_2-receptor antagonist research. Since then, a lot of H_2-receptor antagonist compounds have been synthesized using N^{α}-guanylhistamine as the 'lead' molecule. The approach used to develop second generation histamine H_2-receptor antagonist was the same as the one commonly used by medicinal chemists and pharmacologists to develop new therapeutic agents or to improve the pharmacological or therapeutic profile of a drug or a class of drugs. Certain functional groups of N^{α}-guanylhistamine, the 'lead' molecule, were altered to produce active derivatives; a systematic study of the structure-activity relationships (SARs) of a number of the derivatives were done, which revealed the features which were responsible for alteration or improvement of the activity of the analogues over that of the parent compound, cimetidine. Several studies described the SARs of H_2-receptor antagonists (Ganellin, 1982; Brown and Young, 1985; Ganellin, 1985; Buschauer et al., 1989).

Because of the diverse development of the different chemical derivatives of the histamine H_2-receptor antagonist, quite a few compounds bear little or no structural similarities to their lead compound, N^{α}-guanylhistamine. Despite considerable diversity, the actual known histamine H_2-receptor antagonists can be divided into two main structural classes, compounds containing a flexible connecting chain and compounds with a diaryl structure (Ganellin, 1985; Buschauer et al., 1989).

The majority of the second generation of histamine H_2-receptor antagonists synthesized in recent years tend to be compounds

possessing a flexible connecting chain. The common structural features of this group of compounds are an aromatic ring system, a flexible connecting chain and a polar (uncharged at physiological pH) planar group (Figure 1.3; pp. 22). Modifications to the flexible side chain, such as chain branching or replacing the sulfur atom by alternative heteroatom linkages, resulted in considerable reduction in activity (Brown and Young, 1985; Buschauer et al., 1989). Thus different structural alterations were subsequently aimed at the attachments at both ends of the flexible side chain, the aromatic ring at one end and the polar planar group at the other. In the last decade, research interests were directed to modifications of the Het moiety end of the flexible connecting chain. A lot of compounds had thus been synthesized and tested. Marketed histamine H_2-receptor antagonists, cimetidine and ranitidine are two derivatives within this group. According to the different substituted Het rings, the developed compounds in this group can be subdivided into four main series : a) imidazole, b) aminomethylfuran, c) guanidinothiazole and d) aminomethylphenoxy series.

In recent years, the research interest for further structural substitutions has shifted to the polar group, the other end of the flexible connecting chain. Besides cyanoguanidines, nitroethenediamines, and some other traditionally used polar groups, many novel polar groups, such as "urea equivalents", have been incorporated into antagonist molecules. This included thieno-, benzoisothiazole-1,1-dioxide, isomeric thiadiazole diamines, oxadiazole diamines, diaminopyrimidines, diaminofurazan, and amines such as the

nicotineamide-N-oxide derivative roxatidine (Sorba et al., 1985; Buschauer et al., 1989). The later compound was reported to be a competitive H_2-receptor antagonist about as potent as ranitidine, with a somewhat longer duration of action.

"Urea equivalent" groups are amino-substituted π-electron systems, possessing highly polar and weakly amphoteric moieties with high dipole moments and low octanol-water partition coefficients (Ganellin, 1982). The discovery of novel "urea equivalent" groups is one of the research objectives in the H_2-receptor antagonist field because they can influence the affinity for the receptor, the specificity, the duration of action and the metabonism of the antagonists (Sorba et al., 1985). Histamine H_2-receptor antagonists with cyclic "urea equivalents" have been well studied. Compounds where the aromatic ring (Het) was a pyridyl ring system and the polar group was a 2-nitro-1,1-diaminoethene moiety, a N-alkyl (cycloalkyl) thiourea, a cyanoguanidine moiety, a 3,4-amino-1,2,5-thiadiazole-1-oxide, or a 1,1-dioxide "urea equivalent" Y were reported to be histamine H_2-receptor antagonists (El-Badry et al., 1985; El-Badry et al., 1985b; Agrawal et al., 1987). The results of these studies suggested that the charge distribution in the Het pyridyl ring was important for interaction with the histamine H_2-receptor. It was, therefore, of interest to examine cyclic "urea equivalents" provided by the incorporation of some of the above structures into substituted 1,4-dihydropyridyl, 3-aminopyridyl, 2-amino-3-nitropyridyl, or 2-amino-3-nitro-1H-pyridyl-6-one as the Y component.

The present study revealed that derivatives of cimetidine and

ranitidine with a pyridyl or a 4-methyl-5-imidazolinyl ring at the Het end and a substituted 1,4-dihydropyridyl, a substituted 2-amino-3-nitropyridyl, a substituted 3-aminopyridyl, or a 2-amino-3-nitro-1H-pyridyl-6-one at the Y end of the flexible connecting chain, had lower H_2-receptor antagonist potencies. The most potent compound in this series, VJ-11, was about half as potent as cimetidine, which indicated that the above substitutions were not appropriate modifications for enhancement of histamine H_2-receptor antagonist activity. Some SARs, to be useful for future investigations, were found from the results.

For derivatives with a pyridyl Het moiety and substitutions at the R and X positions of the 1,4-dihydropyridyl Y, the point of attachment of the pyridyl ring at the Het end had an effect on antagonist activity when the remaining structures were similar. It was found that 2-pyridyl analogues were more active than 3-pyridyl and 4-pyridyl derivatives. The relative activity order of 2-pyridyl > 3-pyridyl > 4-pyridyl was in agreement with the data of El-Badry and co-workers (1985; 1985b) who showed that thiourea, cyanoguanidine, and 1-nitro-2,2-diaminoethene "urea equivalents", which also have isomeric pyridyl rings attached, had the same order of potency. These data suggest that charge distribution in the aromatic pyridyl ring may influence the interaction of the H_2-receptor antagonist with the H_2-receptor. The electron density was C-2 < C-4 < C-3 for the pyridyl ring (El-Badry et al., 1985b). Affinity for the H_2-receptor could therefore be due to co-operative and dependent interactions of the molecular substructures of the H_2-receptor antagonist with the H_2-receptor

(Agrawal et al., 1989). The data also suggested that R substitution at the C-4 position of the 1,4-dihydropyridyl ring of the Y end was a determinant of antagonist activity. The relative potency order of the R substituents generally was n-Bu > Ph > Me. It has been hypothesized that NCN and related moieties may interact with H_2-receptor by hydrogen bonding and the strength of this interaction is dependent on the dipoles' ability to align with the receptor. Within this series, a NCN or $CHNO_2$ moiety at the X position did not affect the antagonist activity.

It has been proposed that dipole-assisted hydrogen-bonding may be important to the antagonist-receptor interaction (Agrawal et al., 1988). Since antagonist activity is related more to dipole orientation, rather than its magnitude, the cyclic "urea equivalent" may have an orientational function rather than being involved in a direct dipole-dipole or dipole-charge interaction at the receptor. The 3-nitro group of 2-amino-3-nitropyridyl and 2-amino-3-nitro-1H-pyridyl-6-one is expected to be a determinant of the electron density on the C-2 NH group, thereby influence its ability to hydrogen-bond to an acceptor group on the H_2-receptor. The existence of this type of intramolecular hydrogen bonding was confirmed in the case of ranitidine by spectroscopic study.

For derivatives possessing a pyridyl or a 4-methyl-5-imidazolinyl Het moiety and a substitution at the R position of the 2-amino-3-nitropyridyl Y, the point of attachment of the pyridyl substituent at the Het end also affected the antagonistic activities in an opposite fashion to the above substitutions at the Het end. The order of potency of 4-pyridyl > 3-pyridyl > 2-pyridyl was

inconsistant with the data of Agrawal and colleagues (1987) who showed that 1,2,5-thiadiazole "urea equivalents", which also have different isomeric pyridyl rings attached, had the same order of potency. This again suggests that the affinity for the H_2-receptor is dependent on co-operative interactions of the molecular substructures (pyridyl ring, "urea equivalent"). R substitution on the 2-amino-3-nitropyridyl ring was also a determinant for activity. The relative potency order of the R substituent generally was OMe > H > NH_2 > Cl. The difference between the activity of 4-pyridyl and 4-methyl-5-imidazolinyl Het substitutions were small.

For derivatives possessing a pyridyl Het moiety and a substitution at the X position of the 3-aminopyridyl or a 2-amino-3-nitro-1H-pyridyl-6-one Y, a dramatic decrease of activity was observed. The compounds in this group were more than two orders of magnitude less potent than cimetidine. A C-6 oxo group on 2-amino-3-nitro-1H-pyridyl-6-one moiety is thought to decrease H_2-receptor activity by altering the direction of the dipole and/or by decreasing lipophilicity of the compounds (Agrawal et al., 1988)

5.3 Mechanisms of action of VJ-37 antagonism on antagonist-induced responses in guinea pig right atrium and in GPILSM

The novel pyridyl derivatives of cimetidine discussed above all noncompetitively antagonized the histamine H_2-receptor mediated positive chronotropic response in guinea pig right atrium. This is

interpreted to mean that these compounds did not act directly at the histamine H_2-receptor. Theoretically, noncompetitive antagonism is defined as binding of the antagonist to a site distinct from the agonist-receptor recognition site, which prevents agonist activation of the receptor through allosteric or other mechanisms (Kenakin, 1987b). The nature of noncompetitive inhibition by novel derivatives of cimetidine in this thesis suggested a different mechanism of action in guinea pig right atrium. In attempting to explore the possible mechanisms of action of these compounds, one of the most potent compounds, VJ-37, was further evaluated because of its stability in solution.

Since VJ-37 noncompetitively antagonized the histamine-induced positive chronotropic response, it was interesting to see its effect on other positive chronotropes. The known positive chronotropics are generally believed to either act on receptors or channels on the SA cell membrane, or act on intracellular sites of the SA cells; mobilize Ca^{2+} both extracellularly and intracellularly, leading an increase of cytoplasmic Ca^{2+} which in turn activates contractile proteins and thus accelerates the contractile rate. In this study, the possible sites of action of VJ-37 on either plasma membrane receptors, channels, or intracellular sites were explored further.

At the plasma membrane level, apart from the H_2-receptor agonist histamine, the β-receptor agonist isoproterenol and the Ca^{2+} channel agonist Bay K 8644 are also known to be positive chronotropes. In the present experiments, both isoproterenol and Bay K 8644 induced dose related positive chronotropic effects. It was shown that VJ-37 noncompetitively antagonized the positive

chronotropic effects of isoproterenol. Because Bay K 8644 could not be washed out of the guinea pig right atrium, the characters of inhibition of VJ-37 on voltage sensitive Ca^{2+} channels could not be observed.

In order to clarify the effects of VJ-37 on voltage dependent Ca^{2+} channels and on receptor operated Ca^{2+} channels, the $CaCl_2$- and the muscarinic receptor agonist carbachol-induced tonic contractions of GPILSM were further investigated. The results also indicate that VJ-37 is a noncompetitive inhibitor of the tonic contractions induced by either $CaCl_2$ or carbachol in the GPILSM. While atropine, a muscarinic receptor antagonist, competitively antagonized the effect of VJ-37 in GPILSM. These results clearly showed that VJ-37 did not act directly on the muscarinic receptor, however, the possibility of an action on the voltage sensitive Ca^{2+} channels in the plasma membrane remains to be further considered.

Looking at the effect of VJ-37 intracellularly, the signal transduction pathways of the above receptors and channels in guinea pig right atrium were followed (McNeill, 1980; Tsien, 1987). The first possible intracellular site considered involved cyclic AMP. Cyclic AMP is a second messenger to transfer and amplify signals that come from receptors and channels on the plasma membrane, such as the histamine H_2-receptor or β-adrenergic receptor. Forskolin, an adenylate cyclase activator, increases intracellular cyclic AMP levels indirectly by enhancing the production of cyclic AMP. Milrinone, a PDE inhibitor, also increases intracellular cyclic AMP levels indirectly by retarding the hydrolysis of cyclic AMP.

8-Bromo-cyclic AMP, a permeable analogue of cyclic AMP directly increases intracellular cyclic AMP levels, mobilizes Ca^{2+} both extracellularly and intracellularly by releasing Ca^{2+} from SR and opening voltage sensitive Ca^{2+} channels on plasma membrane. Forskolin, 8-bromo-cyclic AMP, and milrinone all induced dose related positive chronotropic responses. In each case, the positive chronotropic DRCs of 8-bromo-cyclic AMP and milrinone were noncompetitively inhibited. This implies that VJ-37 dose not act specifically at the cyclic AMP level. The forskolin response could not be recovered by repeated washing of the tissue and thus the subsequent DRC could not completed.

The second possible intracellular site of action examined was the caffeine sensitive release of Ca^{2+} from SR. Caffeine had a dose related response in guinea pig right atrium. Once again, VJ-37 had a noncompetitive inhibitory effect on the DRC of caffeine.

As a consequence, the experiments which had been carried out suggest that VJ-37 does not act directly on cell membrane receptors known to be involved with the chronotropic response, nor does it act at the cyclic AMP or SR levels intracellularly. The possibility remains that VJ-37 may affect Ca^{2+} channel activity or act at a point beyond the points investigated along the signal transduction pathways, such as, calmodulin, contractile proteins, Ca^{2+} resequestration, or Ca^{2+} extrusion (Triggle and Janis, 1987). VJ-37 may also act at some other receptors or receptor subtypes, even some other enzymes or pumps.

5.4 Possibility of therapeutic application of VJ-37 as an antigastric acid secretion agent

In spite of the fact that the mechanism of action of VJ-37 did not directly implicate its inhibition of on the histamine H_2-receptor, it was shown that the compound antagonized the histamine H_2-receptor-mediated chronotropic response in guinea pig right atrium; and the data from the pylorus-ligated rat experiments also showed that VJ-37 inhibited rat gastric acid output in vivo. Rat basal gastric acid output was significantly inhibited by VJ-37. Histamine-stimulated gastric acid secretion was not significantly increased relative to that of control animals, because of the large standard error of the histamine-stimulated group.

It was interesting to note that the inhibitory potency of VJ-37 on the histamine-induced chronotropic effect in guinea pig right atrium and on the histamine-induced gastric acid secretion in the pylorus-ligated rat was different. On guinea pig right atrium, VJ-37 was about 3.7 times less potent than cimetidine; while in pylorus-ligated rat, VJ-37 had the same potency as cimetidine. It seemed as if there was an organ specific selectivity for VJ-37 antagonism between the two tissues. VJ-37 and other analogues could therefore be considered for possible therapeutic application in hyper-gastric acid secretion related diseases.

At the subcellular level, certain aspects of drug-receptor interaction and drug disposition could determine the organ selectivity of drugs. The production of a drug response can be

separated into three basic processes (Kenakin, 1987c). The first is the delivery of drug to the receptor compartment. This depends on whether or not there are removal processes (i.e., uptake or metabolic degradation processes) for the drug and the relative ease of permeation into the receptor compartment. The second is the drug-receptor interaction. This can be complicated if the drug interacts with other receptors to modify functionally the primary response. The third (for agonists only) is translation of the receptor stimulus into a tissue response, a process determined by cellular factors of receptor density and the efficiency of stimulus-response coupling. Interference with one or more of these processes could result in synergy or self-cancellation of effects that could in turn result in organ selectivity.

The different antagonist potencies of VJ-37 in different tissues can be explained by drug-receptor interaction. In guinea pig right atrium, exogenous histamine increases heart rate only by stimulating the histamine H_2-receptor. Thus the fact VJ-37 noncompetitively antagonizes the histamine-induced positive chronotropic response only means its nonspecific inhibition at the H_2-receptor. However, the situation was different for rat gastric acid secretion. Theoretically, not only are three receptors involved in the regulation of gastric acid secretion (histamine, cholinergic and gastrin pathway), but also an interdependence exists between these pathways in all phases of acid secretion (Soll and Berglindh, 1987). The finding that VJ-37 noncompetitively antagonized the carbachol-induced tonic contraction of in GPILSM provided evidence that VJ-37 could antagonize muscarinic receptors in GPILSM. This

implies that VJ-37 may also antagonize the muscarinic receptor on the gastric parietal cell membrane. In other words, VJ-37 can antagonize not only histamine H_2-receptor mediated gastric acid secretion which was showed directly by the data of this thesis, but also muscarinic M_1-receptor mediated gastric acid secretion. The enhanced antagonist potency of VJ-37 in the pylorus-ligated rat suggests that inhibitory synergisms and possibly interactions of the receptors involved in the gastric acid secretion may be involved.

The organ specific selectivity of VJ-37 antagonism in different animal models also indicates that although the guinea pig right atrial assay is a good model for histamine H_2-receptor antagonist screening, it might not be if the screened compounds noncompetitively antagonize the histamine-induced positive chronotropic response. The major medical therapeutic application of H_2-receptor antagonists is to inhibit gastric acid secretion. For compounds which noncompetitively inhibit the histamine-induced positive chronotropic response in guinea pig right atrium, their inhibitory potencies may different in the *in vivo* gastric acid secretion experiment because of possible receptor synergisms and interactions involved in gastric acid secretion. In this situation, the pylorus-ligated rat model may be one of the best animal model available.

CHAPTER VI

CONCLUSIONS

In attempting to design novel histamine H_2-receptor antagonists, a new class of pyridyl derivatives of cimetidine and ranitidine with the general structure of Het-$CH_2SCH_2CH_2$-Y has been synthesized, where Het is a pyridyl or a 4-methyl-5-imidazolinyl Het ring system and Y is a substituted "urea equivalent". These compounds were evaluated as histamine H_2-receptor antagonists by using the histamine-induced positive chronotropic response in guinea pig right atrium in vitro.

The data revealed that for derivatives of cimetidine with a pyridyl or a 4-methyl-5-imidazolinyl Het ring in connection with a substituted 1,4-dihydropyridyl, a substituted 2-amino-3-nitropyridyl, a substituted 3-aminopyridyl, or a 2-amino-3-nitro-1H-pyridyl-6-one "urea equivalent Y" by a flexible side chain, the antagonist potencies were generally decreased. This indicated that the above substitutions were not the appropriate alterations for enhancing antagonist activity. The most potent compound in this series, VJ-11, was about half as potent as cimetidine. The structure-activity relationships of these compounds were then analysed.

For derivatives with a pyridyl Het ring and substitutions at the R and X positions of the 1,4-dihydropyridyl Y, the point of

attachment of the pyridyl ring at the Het end affected antagonist activity. The relative potency order was 2-pyridyl > 3-pyridyl > 4-pyridyl. R substituents at the C-4 position of the 1,4-dihydropyridyl ring at the Y end were found to affect the antagonist activity. The relative potency order of R substituents generally was n-Bu > Ph > Me. Introducing either a NCN or a $CHNO_2$ moiety at the X position did not affect antagonist activity within this series.

For derivatives possessing a pyridyl or a 4-methyl-5-imidazolinyl Het moiety and a substitution at the R position of the 2-amino-3-nitropyridyl Y, the point of attachment of the pyridyl ring at the Het position affected antagonist activity. The potency order was 4-pyridyl > 3-pyridyl > 2-pyridyl isomers, which was opposite to the above results. The R substituent on the 2-amino-3-nitropyridyl ring was also a determinant for activity. The relative potency order generally was Ome > H > NH_2 > Cl. Moreover, the difference between activities of 4-pyridyl and 4-methyl-5-imidazolinyl Het substituents was small.

For derivatives possessing a pyridyl Het moiety and a substitution at the X position of the 3-aminopyridyl or a 2-amino-3-nitro-1H-pyridyl-6-one Y, a dramatic decrease of activity was obtained. The compounds in this group were more than two hundred times less active than cimetidine.

The nature of the novel derivatives of cimetidine and ranitidine on the histamine induced positive chronotropic response in guinea pig right atrium was noncompetitive, suggesting a different mechanism of action other than H_2-receptor antagonism. In order

to explore the possible mechanisms of action of these compounds, one of the most potent compounds, VJ-37, was further evaluated. The antagonism of the positive chronotropic response of isoproterenol, 8-bromo-cyclic AMP, milrinone, and caffeine in guinea pig right atrium and the tonic contractile responses of $CaCl_2$ and carbachol by VJ-37 were also noncompetitive in nature. These data suggest that VJ-37 does not act through a mechanism involving the histamine H_2-receptor, the β_1-adrenergic receptor, the muscarinic M_1-receptor, intracellular cyclic AMP, or by inhibiting the release of intracellularly stored SR Ca^{2+}. The possibility of an action on the voltage sensitive or receptor-operated Ca^{2+} channels remains to be further investigated.

No matter what kind of mechanism of action VJ-37 had, it inhibited both basal and histamine-induced gastric acid secretion in the pylorus-ligated rat, with the same potency as cimetidine. While in the guinea pig right atrial assays, it was about 3.7 times less active as cimetidine. The tissue selectivity of VJ-37 antagonism is proposed to be due to possible receptor synergisms and interactions among different receptors, such as the histamine H_2-receptor, the muscarinic M_1-receptor, and the gastrin receptor.

CHAPTER VII

FUTURE CONSIDERATIONS

Successful treatment of peptic ulcer disease by first generation histamine H_2-receptor antagonist has provided the stimulus to develop second generation histamine H_2-receptor antagonists. The desirable compounds should be more effective, exhibit a longer duration of action, and possesses no fatal side effect. In recent years, a lot of novel compounds had been synthesized and screened; clinical trials have been completed on quite a few of them. Because of a variety of toxicities found in those compounds, only a few compounds are still considered to do further clinical trails, such as famotidine, nizatidine (Weir, 1988). However the achievements have given us a better understanding of the structure of histamine H_2-receptor and the structural requirements for histamine H_2-receptor antagonists (Buschauer et al., 1989). The present data suggest that the derivatives of cimetidine which possess a pyridyl or a 4-methyl-5-imidazolinyl Het ring and a substituted 1,4-dihydropyridyl, a substituted 2-amino-3-nitropyridyl, a substituted 3-aminopyridyl, or a 2-amino-3-nitro-1H-pyridyl-6-one "urea equivalent Y" are not the appropriate structural modifications for increasing histamine H_2-receptor antagonist activity. Further modifications based on these structures should not be considered.

Exploration the mechanisms of action of these novel compounds,

particularly for VJ-37, should receive special attention because of their noncompetitive antagonisms on histamine H_2-receptor-induced chronotropic response in guinea pig right atrium. Further biological experiments, such as skinned fibre preparation, are needed to study the possible action sites of VJ-37 along the signal transduction pathways which we did not investigate, such calmodulin or contractile proteins. Additional biochemical investigations, such as radioligand binding studies are also required to provide another piece of evidence to clarify the action of VJ-37 on receptors or channels of the cellular membranes. It would be very helpful to study the electrophysiological effects of VJ-37, in order to better understand the mechanisms of action of it.

The data of the in vivo gastric acid secretion experiment of the VJ-37 also remind us to reconsider the possible antigastric acid secretory effect of some potent compounds in this thesis, especially for VJ-11 which was about half as potent as cimetidine in guinea pig right atrium assays. VJ-11 may be more potent than cimetidine to inhibit rat gastric acid secretion because of the possible receptor synergisms and interactions among different receptors like VJ-37 possesses.

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